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MRI Project No. 4513-B

MAMMALIAN TOXICOLOGICAL EVALUATION OF RDX

Final Report

September 1980

Supported By

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-78-C-8027

Contract Officer's Technical Representative:
Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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RDX
Hexahydro-1,3,5-trinitro-1,3,5-triazine
CAS Reg. No. 121-82-4
Acute toxicity

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Acute oral LD_{50}'s and standard errors were 119.0 $\pm$ 4.6, 118.7 $\pm$ 4.5, and 118.1 $\pm$ 2.8 mg/kg in male, female and combined sexes of rats. The corresponding mouse LD_{50}'s were 97.2 $\pm$ 8.7, 58.9 $\pm$ 26.8, and 80.3 $\pm$ 9.6 mg/kg, respectively. There were no statistically significant sex differences in either species. Toxic signs, including gasping, labored breathing and convulsions, indicated neurotoxicity.

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19. (concluded)

Subchronic (90-day) toxicity
Ames Salmonella/microsome test
Dominant Lethal Mutation Study
Teratology
Reproductive toxicity
Rats
Mice
Rabbits

20. (concluded)

In the 90-day rat subchronic toxicity study, 40 mg/kg/day was toxic, while the next lower dose (28 mg/kg/day) was not. The only consistent toxic effect observed was decreased weight gain and (in males) decreased feed consumption in some weeks.

Mice were less affected in the 90-day subchronic study, since a dose of 320 mg/kg/day was required to produce definite toxicity. Effects seen in one or both sexes included hyperactivity, unscheduled deaths, and increased liver and kidney weight accompanied by hepatocellular vacuolization or microgranulomas and tubular nephrosis.

RDX was not mutagenic in the Ames Salmonella/microsome test at doses up to 1 mg/plate and in the rat dominant lethal mutation test at doses up to 50 mg/kg/day.

RDX was not teratogenic to rats or to rabbits at doses up to 20 mg/kg/day but at that dose produced severe nonteratogenic toxicity in rats. No adverse effects were observed at 2 mg/kg/day.

The two-generation reproduction study produced severe toxicity (particularly neurotoxic effects and unscheduled deaths) but no specifically reproductive toxicity at diets giving a nominal 50 mg/kg/day. Feeding 16 mg/kg/day produced no apparent effects.

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MRI Project No. 4513-B

MAMMALIAN TOXICOLOGICAL EVALUATION OF RDX

Final Report

By

J. M. Cholakis
L. C. K. Wong
D. L. Van Goethem
J. Minor
R. Short
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H. V. Ellis III

September 1980

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Fort Detrick, Frederick, Maryland 21701

EXECUTIVE SUMMARY

In order to define the mammalian toxicology of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine; CAS Reg. No. 121-82-4), a variety of studies were carried out. Classical toxicity studies included acute oral lethality and subchronic (90-day) feeding studies in rats and mice. Mutagenicity studies included the Ames Salmonella/microsome test and a dominant lethal mutation study in rats. Reproductive toxicity studies included teratology studies in rats and rabbits and a two-generation reproductive study in rats.

Acute oral LD_{50}'s and standard errors were 119.0 ± 4.6, 118.7 ± 4.5, and 118.1 ± 2.8 mg/kg in male, female and combined sexes of rats. The corresponding mouse LD_{50}'s were 97.2 ± 8.7, 58.9 ± 26.8, and 80.3 ± 9.6 mg/kg, respectively. There were no statistically significant sex differences in either species. Toxic signs, including gasping, labored breathing and convulsions, indicated neurotoxicity.

In the 90-day rat subchronic toxicity study, 40 mg/kg/day was toxic, while the next lower dose (28 mg/kg/day) was not. The only consistent toxic effect observed was decreased weight gain and (in males) decreased feed consumption in some weeks.

Mice were less affected in the 90-day subchronic study, since a dose of 320 mg/kg/day was required to produce definite toxicity. Effects seen in one or both sexes included hyperactivity, unscheduled deaths, and increased liver and kidney weight accompanied by hepatocellular vacuolization or microgranulomas and tubular nephrosis.

The estimated maximum tolerated dose (MTD) for male rats is 50 mg/kg/day, but somewhat higher for females. The MTD for mice is less than 320 mg/kg/day but greater than 40 mg/kg/day.

RDX was not mutagenic in the Ames Salmonella/microsome test at doses up to 1 mg/plate and in the rat dominant lethal mutation test at doses up to 50 mg/kg/day.

RDX was not teratogenic to rats or to rabbits at doses up to 20 mg/kg/day. That dose did produce severe nonteratogenic toxicity in rats. oN adverse effects were observed at 2 mg/kg/day.

The two-generation reproduction study produced severe toxicity (particularly neurotoxic effects and unscheduled deaths) but no specifically reproductive toxicity at diets giving a nominal 50 mg/kg/day. Feeding 16 mg/kg/day produced no apparent effects.

FOREWORD

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), Fort Detrick, Frederick, Maryland, has been conducting a research program since 1973 for the purpose of developing the scientific data base from which water quality criteria for compounds unique to the munitions industry could be determined. A water quality criterion (as defined by the amended Clean Water Act, 1977) is a qualitative or quantitative estimate of the concentration of a pollutant in ambient waters that, when not exceeded, will ensure a water quality sufficient for a specified water use. The criterion is a scientific entity based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility. Currently, a water quality criterion consists of two separate numerical limits, one for the protection of human health and the other for the protection of aquatic organisms. These numbers, when translated by the appropriate regulatory agency, can be the basis of enforceable discharge or effluent limitations in a point source discharge permit issued under the Clean Water Act.

Since a water quality criterion is to protect designated water uses, a diverse, multidisciplined research program was developed by USAMBRDL that includes "effects" studies on laboratory and domestic animals, wildlife species, aquatic organisms, plants, and economically important crops. In addition, extensive chemical and biological fate and persistence tests are conducted to provide information on the behavior of a pollutant in the aqueous environment. These kinds of data are especially useful for making site-specific translation of criteria into enforceable discharge limits.

This report represents a portion of the mammalian toxicology data base being developed by USAMBRDL on materials related to the use and disposal of RDX.

Animal experimentation: Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1978), DHEW Publication No. (NIH) 78-23, prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council; the regulations and standards prepared by the Department of Agriculture, and the Public Law 91-579, "Laboratory Animal Welfare Act" (1970).

Citations of trade names in this report do not constitute an official Department of the Army endorsement or approval of the use of such items.

PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD17-78-C-8027, MRI Project No. 4513-B, entitled "Chronic Mammalian Toxicological Evaluation of RDX." Dr. Jack C. Dacre, Environmental Protection Research Division, U.S. Army Medical Bioengineering Research and Development Laboratory, was the contract officer's technical representative for the project.

The work was conducted between March 1, 1978, and February 29, 1980, in the Pharmacology/Toxicology Department of the Chemical and Biological Sciences Group, Dr. Florence I. Metz, Acting Director, succeeded by Dr. Thomas E. Shellenberger, Director. Dr. Laurence C. K. Wong, succeeded by Dr. H. V. Ellis III, was Principal Investigator for the project. Dr. James M. Cholakis was study director for the Toxicology studies. He was assisted by Mr. John J. Kowalski, Miss Diana Ungerman, Mr. Steven Unwin, Mr. Jack Hagensen, Mr. Darrel Lavish, Mrs. Pam Lavish and Mrs. Karen C. Smith. The Ames test was done by Mr. Daniel L. Van Goethem. Mr. Jan Minor, succeeded by Dr. Robert Short, was study director for the dominant lethal mutation and reproductive studies, assisted by Mr. Timothy Unger, Mr. Bradley Breeden, and Mr. Daniel L. Van Goethem. Supporting work included analytical chemistry by Dr. Danny O. Helton of the Analytical Chemistry Department, with the assistance of Mr. Tom Kuntz and Mrs. Gail K. Rehagen; clinical chemistry by Miss Ilonna Elwood (supervisor) and Mr. Duane Smith; prosection by Mr. Ernesto A. Castillo (supervisor), Miss Mary Lee Carr and other project personnel, histochemical preparation of tissues by Mrs. Ellen R. Ellis (supervisor), Mrs. Janet Kleithermes, and Mr. Kerry Crabb; and histopathologic examination of tissues by Dr. Chuen-Bin Hong and Dr. Helmuth Sprinz.

MIDWEST RESEARCH INSTITUTE

Harry V. Ellis III

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Senior Toxicologist

Approved:

Thomas E. Shellenberger

Thomas E. Shellenberger
Director, Pharmacology/Toxicology Department

September 10, 1980

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I. INTRODUCTION

RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine; CAS Reg. No. 121-82-4; cyclotrimethylenetrinitramine; cyclonite; hexogen) is a high explosive used in filling shells and bombs (commonly in a 60-40 mix with trinitrotolulene) and in demolition charge (commonly as composition C-4). Despite its wide-spread military use, only limited toxicological data, by modern standards, are available. Therefore these studies were done to check the few reported data and to expand our knowledge of the mammalian effects of RDX. These studies are divided into three groups: classic toxicology (acute oral lethality and subchronic feeding studies in rats and mice), mutagenicity (Ames Salmonella/microsome test and dominant lethal mutation study in rats), and reproductive toxicity (teratology studies in rats and rabbits and a two-generation reproductive study in rats).

This report consists of a general methods section, sections on the results of the various studies, and a general discussion and conclusions.

II. MATERIALS AND METHODS

A. RDX

1. Bulk compound: All studies were done with portions of a single lot of RDX, No. HOL 43537, supplied by Holston Army Ammunition Plant, Kingsport, Tennessee. The material was composed of small, yellowish-tan flakes. This lot was found to contain 2.2 ± 0.1% water, 88.6 ± 0.9% RDX, and about 9% HMX. No other compounds were detected; the limit of detection was estimated as < 0.5% of total material. See the reports in Appendix I for details.

2. Dose preparation:

a. Suspensions: Before preparation of the suspension, bulk RDX was crushed by hand. A concentrated suspension was prepared by hand mixing and diluted to proper concentrations with magnetic stirring. The vehicle was either 1% methylcellulose (E50 Premium, Lot No. QP-212376-E, or a 1:1 mixture of E50 Premium, Lot No. QP-320471-E, and K4M Premium, Lot No. MM-040481-K, all from Dow Chemical Co.) in water ("MC") or 1% methylcellulose and 1% polysorbate 80 (Tween 80®, Lot No. 766613 or Lot No. 781666, Atlas Chemical Industries) ("MCTW") in water. Maintaining uniform suspensions was not always easy. Freeze-thaw cycles helped, especially with the more concentrated suspensions.

b. Solution: For the Ames test, RDX was dissolved, at appropriate concentrations, in dimethylsulfoxide.

c. Feed mixes: Before mixing, RDX was ground in a ball mill to about 200 μm particle size, similar to that of the feed. Size was monitored with an American Optical Spencer Hemocytometer. This ground RDX was mixed with laboratory rodent chow to provide successive dilutions of 10% and 1% RDX in feed. In most cases these were further diluted to provide stock mixtures which were diluted weekly to provide the actual feed mixtures.

Mixing was accomplished using a converted cement-type box mixer. The mixer was located under a hood that could be turned on automatically with the mixer switch. Prior to any mixing of dose levels, the RDX-labeled wooden box was brushed clean; then approximately 300 g of control feed was added to the box and allowed to mix for 10 min. This feed was discarded. The lowest concentration (or mg/kg dose) was always mixed prior to the higher doses. The appropriate control feed, previously weighed, was added to the box, then the appropriate amount of stock from a beaker or weighing dish. The beaker or dish was "rinsed" clean twice using the control feed in the box. Each dose level was allowed to mix 30 min. When mixing was complete, the feed was poured back into the plastic feed bucket and the mixing box was brushed clean of any adhering feed. The closed feed buckets were then transported to the main facility for feeding.

3. Dose assays: A variety of dosage preparations were assayed for RDX content. Some of these were trial mixes to develop methods and check stability of the preparations. Others were actual dosage preparations, constituting spot checks, rather than a systematic assay. Methods and results are detailed in Appendix I.

Most assays found the samples to be more concentrated than nominal; the average ± standard deviation of all feed samples was 129 ± 26% of nominal. Suspensions were more variable than feed samples. Repeated samples of the same nominal concentration varied widely, implying that the situation consists of random variation rather than a systematic error.

Nominal concentrations of RDX were used in all studies described below. Since the actual concentration was somewhat higher (especially in the feeding studies), conclusions on toxic and nontoxic levels will have a safety factor due to the stronger than nominal concentrations used and to the HMX content of the RDX.

B. Animals

1. Procurement: Adult Fischer 344 rats and B6C3F1 hybrid mice were bought from Charles River Breeding Laboratories (North Wilmington, Massachusetts) for the toxicology studies. CD® rats from the same supplier were used for the dominant lethal mutation and two-generation studies. New Zealand rabbits were bought from Small Stock Industries, Pea Ridge, Arkansas.

All animals were quarantined for at least one week before use.

2. Husbandry: Animals were usually gang housed in plastic shoebox cages with corn cob bedding (Bed-O-Cob®) (rodents) or in metal wire cages (rabbits). Feed and tap water were available *ad libitum*. Feed was Purina Laboratory Chow for rodents or Wayne Lab-Blox (Allied Mills, Inc., Chicago) rabbit chow, except that the mash form of Wayne Lab-Blox was used

for animals given dosed feed (subchronic, dominant lethal mutation and two generation reproductive studies).

Animal rooms were kept at 72° ± 3°F (22° ± 2°C) and 50% ± 10% relative humidity and a 12-hr photo period.

The animals were weighed, stratified by weight into strata containing one animal per test group and then randomly allocated among the test groups and ear-tagged before dosing; tagging was omitted for animals in the acute tests.

C. Toxicology Studies

1. Acute oral toxicity:

a. Dosing: Animals were fasted overnight and given gavage doses of MC (rats) or MCTW (mice) suspensions with a stainless steel dosing needle. Concentrations were adjusted to provide 20 ml/kg doses.

b. Procedures: Rats were dosed when about 3 months old (males: 154 to 213 g; females: 102 to 135 g) and mice when about 2 months old (males: 19 to 22 g; females: 15 to 19 g). After limited range-finding studies, 20 rats and 10 mice (equally divided by sex) were given each of six doses at equal logarithmic intervals. Animals were observed for immediate signs of toxicity or mortality. Subsequently, all survivors were observed daily for 14 days. Gross necropsies were done on representative animals.

The LD_{50}, slopes, 95% confidence limits, and other parameters were calculated by a computer program based on the method of Finney[1].

2. Subchronic toxicity study:

a. Dosing: RDX was administered in dosed feed. The animals were weighed, and feed consumption determined, weekly. From these data, RDX concentrations were calculated to produce RDX intake of 0, 10, 14, 20, 28 or 40 mg/kg/day for each dosage group. A later supplemental study with mice only included four dosage groups fed 0, 40, 60 or 80 mg/kg/day for 2 weeks followed by 0, 320, 160 or 80 mg/kg/day, respectively, for 11 weeks.

b. Procedures: Each dosage group contained 10 males and 10 females (except there were 12 females/group in supplemental study). Animals were put on study at about 5 weeks' age. Starting weights for male rats were 73 to 98 g; for females, 53 to 69 g. Starting weights for the male mice were 20 to 29 g (18 to 22 g in the supplemental study); for the females, 18 to 22 g (16 to 18 g). Animals were observed daily 7 days a week for behavioral changes, toxic signs and other abnormalities , body weight and feed consumption. Abnormal animals (those having behavioral change, marked weight loss or other severe signs) were observed closely and killed for necropsy or when judged moribund. The study was terminated after 13 weeks.

c. Clinical pathology:

(1) Hematology: Hematology determinations included erythrocyte, reticulocyte, white cell, and platelet counts, hematocrit, hemoglobin, methemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and nucleated erythrocyte count. These parameters were evaluated at 30 and 60 days and at termination in five rats per sex from control, 28 and 40 mg/kg/day groups; five mice per sex from control, 28 and 40 mg/kg/day dosage groups at termination only of the main study; and five mice per sex from each dose group in the supplemental study.

(2) Clinical chemistry: The clinical chemistries included serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase, (SGOT), fasting blood glucose, sodium, potassium and calcium electrolytes, blood urea nitrogen (BUN), and alkaline phosphatase. Clinical chemistries parameters were determined in both five rats per sex from the control and the two highest dose levels at termination only. SGOT and BUN were performed on blood obtained via cardiac puncture in five mice from the control and the two highest dose levels at termination only.

d. Necropsy/histopathology: After 90 days on test, all surviving animals were killed using ether anesthesia and necropsied. The following tissues were obtained: adrenals, pituitary, thyroids, esophagus (mouse only), trachea (mouse only), heart, lungs, liver, spleen, gallbladder (mouse only), gonads, pancreas, thymus (mouse only), salivary glands, mesenteric lymph node, stomach, intestines, kidney, urinary bladder, muscle, diaphragm, skin, brain (two sections), bone, eyes, spinal cord, tumors and other lesions. In addition, te following tissues were weighed at necropsy: brain, heart, liver, kidneys, spleen, and gonads. The tissues were then preserved in 10% buffered formalin, trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. With rats, all control animal tissues and tissues from the highest dose level (40 mg/kg/day) were examined microscopically by a veterinary pathologist. No histopathologic examination was performed on mice in the main study; tissues from control mice and high-dose (40 to 320 mg/kg/day) mice were examined in the supplemental study.

e. Statistics: Tukey's omega procedure[2] was used for body weight and feed consumption data, while Dunnett's multiple comparison procedure[3] was used for clinical pathology data.

D. Mutagenicity Studies

1. Ames Salmonella/microsome test: The protocol used for evaluating the mutagenic potential of RDX is described in detail in Appendix II. Briefly, RDX was dissolved in dimethylsulfoxide (DMSO) at concentrations of 10 mg/ml, 3 mg/ml, and 1 mg/ml. Lower doses (100 µg/ml and 10 µg/ml) were prepared by subsequent serial dilution with DMSO. One-tenth milliliter aliquots of these solutions were used to assay RDX at 1,000, 300, 100, 10 and 1 µg/plate. All dose levels were run in duplicate with and without metabolic activation.

The S-9 mix, the metabolic activation system, was freshly prepared. It contained the 9,000 x g supernatant fraction of liver from Aroclor 1254 (500 mg/kg) induced rats (Sprague-Dawley, Charles River Breeding Labs). The bacterial tester strains were obtained from Dr. Bruce N. Ames on September, 1977. The strains are stored at -80°C and fresh cultures were prepared for the RDX assay. Vehicle controls and positive controls were included for each strain. Compounds used for positive controls were included for each strain. Compounds used for positive controls were cyclophosphamide (200 μg in 0.1 ml DMSO per plate, Mead Johnson) for TA-1535 and benzo[a]pyrene (5 μg in 0.1 ml DMSO per plate, Sigma Chemical Company) for TA-1537, TA-1538, TA-98 and TA-100. All control and test plates were incubated at 37°C for 48 hr. Plates were then checked for a normal background lawn and macroscopic colonies were enumerated.

2. Dominant lethal mutation study: This study used F_0 males from the two-generation reproduction study. After the mating of F_0 males and females in that study, the F_0 males were mated with untreated virgin females. This began after about 15 weeks' feeding with RDX. Each male was housed with two females a week for two weeks. These females were killed at midgestation (calculated as the middle of the week of cohabitation) and the number of corpora lutea, implants, live embryos and dead embryos recorded.

All other procedures are as described below for the two-generation reproduction study.

E. Reproductive Toxicity

1. Teratology studies:

a. Mating: One sexually mature female was co-housed with one sexually mature male. With rats, vaginal smears were prepared and examined for sperm and estras; if sperm were seen, the day was designated day 0 of gestation and the male removed. With rabbits, copulation was observed and that day designated day 0.

b. Dosing: Animals were divided into four dosage groups given 0, 0.2, 2.0, or 20 mg/kg/day of RDX in suspensions by gastric intubation. Suspensions for rats were prepared with MCTW vehicle; those for rabbits were from a MCTW stock suspension diluted with MC. A small pilot study was done on rats.

Rats were dosed by gavage on days 6 through 19 of gestation. Rabbits were dosed by gavage on days 7 through 29.

A positive control group of rabbits was given 3 mg/kg/day of 6-aminonicotinamide by gavage for 1 or 4 days starting on day 9; the treatment period was curtailed due to toxicity. The positive control for rats was 350 mg/kg hydroxyurea on day 6 followed by vehicle only on days 7 through 12.

c. Observations: Body weights were recorded on gestation days 6, 7, 9, 13 and 19 in rats and days 7, 14, 21 and 28 in rabbits. Feed consumption was monitored periodically. Dams were monitored daily for toxic signs.

d. Fetal observations: On day 20 (rats) or day 30 (rabbits) of gestation, the dams were weighed, sacrificed and the viscera examined grossly. The uterus and ovaries were removed in toto, and the uterus was than opened and examined for the number and position of live fetuses and resorptions. Resorption sites were noted as early if there was evidence of implantation without a recognizable embryo or fetus or late if a dead embryo or fetus was present with external degenerative changes. All placenta were inspected for abnormalities. The ovaries were examined and the number of corpora lutea counted.

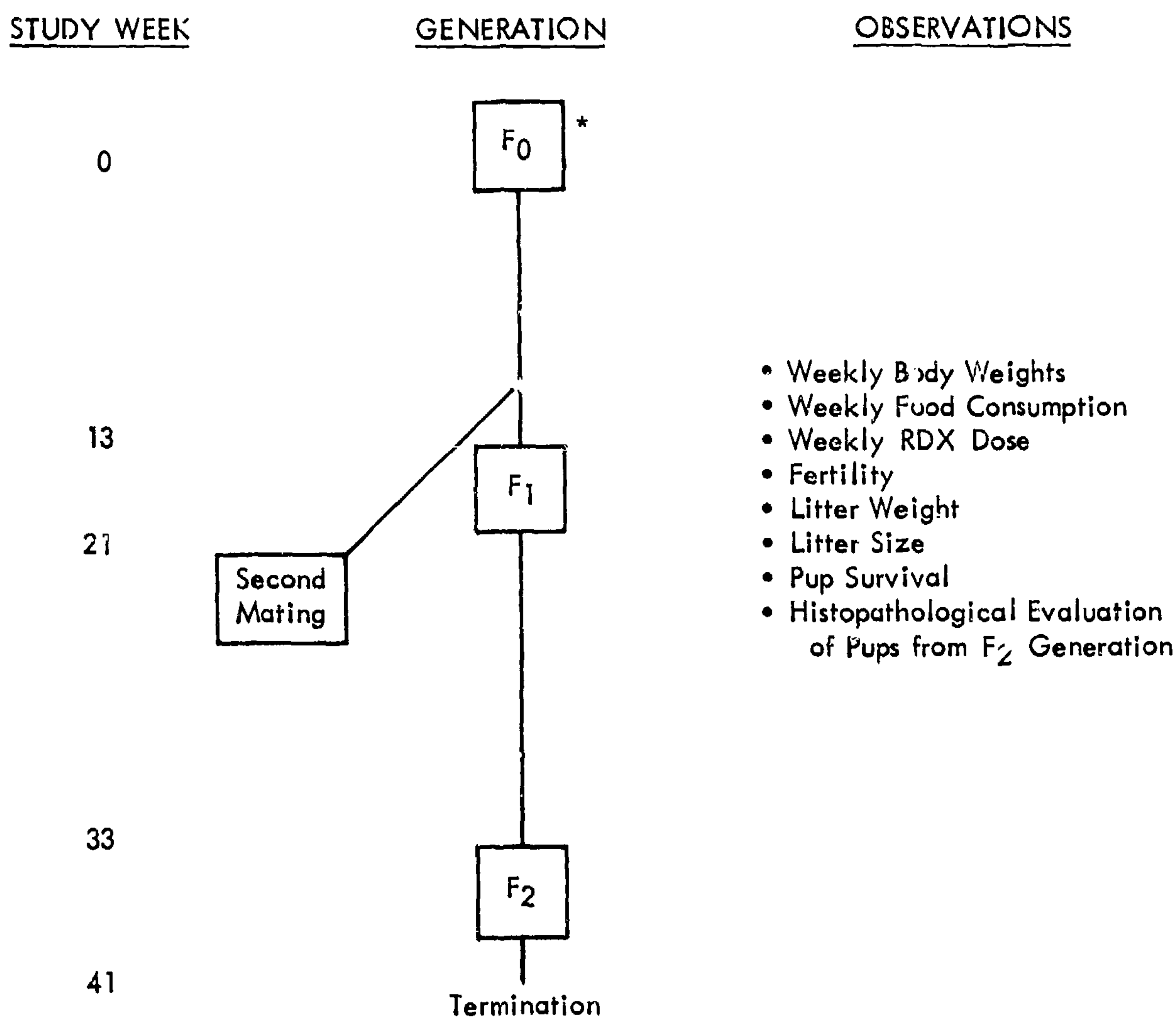
All fetuses were counted, number, weighed and sexed. They were checked for validity and examined for external malformatons as described by Wilson.[4]

Approximately one-half (rabbits) or one-Third (rats) of the viable fetuses from each litter were dissected and examined for soft tissue anomalies by the free-hand slicing method of Wilson.[4] Each fetus was fixed in 20 to 25 ml of Bouins fluid for two weeks. The hardened fetuses were examined for external anomalies and serially cut from the head through the trunk using a sharp razor blade. No slices were made beyond the kidneys, and the intestines were carefully removed from the pelvic cavity. The cross-sections of the fetuses and the genitourinary organs on the pelvic floor were carefully examined by experienced personnel. The remaining viable fetuses from each litter were processed for skeletal examination. Fetuses were fixed in 70% alcohol for two weeks and eviscerated. The fetuses were stored in 1% KOH for two days then stained with alizarin red.[5] After differential decolorization, the skeletons were examined by experienced personnel for anomalies.

e. Statistics: Data were analyzed by a nonparametric rank test.[6] The level of significance was selected as $p < 0.05$ unless otherwise indicated. The litter was considered the experimental unit of observation. For example, the percent of fetuses with a given anomaly was calculated for each litter. These percentages were then analyzed by a nonparametric rank test.

2. Two-generation reproduction study:

a. Outline: An outline of the protocol used in the present study is presented in Figure 1. Four groups, each consisting of 22 male and 22 female rats, were fed continuously diets which contained quantities of RDX to provide nominal daily doses of 0, 5, 16 or 50 mg/kg. In an effort to provide a constant dose of RDX, (a) the concentraton of RDX in the diet was varied at weekly intervals as the animals grew and (b) males and females were fed diets containing different concentrations of RDX. The actual doses of RDX consumed were calculated for males and females during each generation.



* One control and three groups that received diets that contained RDX. The concentration of RDX in the diets was varied in an effort to provide a constant dose of RDX. The actual doses of RDX consumed by the various groups are presented in Tables 60-63.

Figure 1 - Two-Generation Study with RDX

b. F_0 generation: The parenteral generation (F_0) was treated for 13 weeks. During this time, weekly body weights, feed consumption, and RDX doses were calculated. After 13 weeks males and females in each group were co-housed in the ratio of one male to one female. During each day of co-housing, females were examined for sperm-positive vaginal smears. If such evidence of mating was observed, the male was removed to a separate cage, the female was weighed, and the day was identified as day 0 of gestation. Mated females were weighed on days 0, 13, and 20 of gestation. Dams were allowed to deliver, and their pups were counted on days 0, 7, 14 and 21 and weighed on days 0 and 4 after birth and again at weaning. Since adverse effects on reproduction were observed in the high dose group, control and high dose females were also mated with nontreated proven male breeders. In addition, F_0 males were mated with nontreated females to determine if RDX produced a dominant lethal effect. The results of those matings are included separately under mutagenicity studies.

c. F_1-generation: The F_1 generation was weaned, and rats were randomly selected from each litter and maintained on the RDX diets. The control, low, and mid-dose groups each consisted of 26 males and 26 females. In contrast, the high dose group contained only one litter of four males and two females. After weaning, each group was fed the appropriate diet for at least 13 weeks. At the end of this treatment, males and females were mated as previously described.

d. F_2 generation: After the rats were weaned, males and females were randomly selected from litter for necropsy. The tissues listed below were fixed in neutral buffered 10% formalin and processed for histopathological evaluation. All tissues were stained with hematoxylin and eosin.

Skin	Uterus	Pituitary
Trachea	Testicle	Thyroid
Lung	Epididymis	Adrenal
Abdominal Aorta	Prostate	Rib
Heart	Seminal Vesicle	Diaphragm
Spleen	Salivary Gland	Skeletal Muscle
Thymus	Esophagus	Brain
Lymph Node (mes.)	Stomach	Spinal Cord
Kidney	Intestine	Sciatic Nerve
Bladder	Pancreas	Eyes
Ovary	Liver	

e. Statistics: Quantitative data are reported as the mean standard error and were analyzed for statistical significance by Tukey's omega procedure.[2]

III. TOXICOLOGY STUDIES

A. Acute Oral Toxicity in Rats and Mice

The mortality data and pharmaco-toxic signs along with computed LD_{50}'s, slopes, standard errors and 95% confidence limits, are found in Table 1 (rats) and Table 2 (mice).

1. Rats: Within 2-3 hr after RDX administration 100% of the animals died at doses of 250, 180, and 150 mg/kg (at 200 mg/kg 19/20 animals died within 2-3 hr with one animal surviving until termination of study). The mortality rate was 70% and 5% for animals treated at 125 and 100 mg/kg, respectively. There was no apparent sex difference in response to RDX treatment since the mortality rates and the LD_{50} values were essentially equal in both sexes:

LD_{50} (95% confidence limits) in mg/kg

male - 119.0 (110.4 to 128.3)
female - 118.7 (108.0 to 128.9)
combined - 118.1 (111.8 to 124.1)

Details of pharmaco-toxic signs by dosage groups are presented in Table 1, and included predominantly central nervous system involvement, for example, gasping/labored breathing and convulsions. Gross necropsy of animals revealed post-mortem changes not attributable to test compound administration. Computer outputs of probit analysis of mortality data by sex and by male and female rats (combined) are found in Tables 3-5.

2. Mice: Within 5-10 min after RDX administration, 100% of the animals died at doses of 350, 225, and 180 mg/kg RDX. Also, 90% and 60% of the mice died within 30 min at 140 and 100 mg/kg, respectively. At 60 mg/kg three female mice died on day 10 of test. The reason for these delayed deaths is not known. All animals which died (except the three female animals in the 60 mg/kg RDX group) demonstrated predominantly central nervous system signs and included gasping/labored breathing, Straub tail-like symptoms, and clonic/tonic convulsions prior to death. (See Table 2 for details of toxic signs by dosage groups.)

There may be an apparent sex difference in response to RDX treatment when LD_{50} values and mortality rate are compared in both sexes; however, overlapping confidence limits do not support this claim. The LD_{50} values (95% confidence limits) in milligrams per kilogram: male mice, 97.2 (81.6 to 115.8); female mice, 58.9 (24.8 to 139.5); combined for males and females, 80.3 (55.3 to 99.2). Gross necropsy of a representative number of animals did not reveal any lesions attributable to test compound administration. Computer outputs of probit analysis of mortality data by sex and by male and female mice combined are found in Tables 6-8.

B. 90-Day Subchronic Study in Rats

1. Body weight, feed consumption, clinical signs and mortality: Mean body weight gains of the male rats administered 40 mg/kg/day of RDX (highest dose group) in the diet were significantly lower than control values throughout the entire study except for week 2 (Table 9). After 13 weeks on the test, this weight gain was 8.4% lower when compared to the control mean body weight value. In addition, the mean body weight gain was dose-related for all 13 weeks of test.

There were no eventful statistical changes in mean body weight values for female rats during the entire study; however, dose-related lowering of body weight gain was noted after the 13th week of study. The mean body weight gain was 5.5% lower for the 40 mg/kg/day dosage group as compared to the control weight (Table 10).

Feed consumption in male rats fed 40 mg/kg/day of RDX was significantly lower than control animals during 5 weeks. Feed consumption in this dosage group was 17.1%, 10.8%, 13.1%, 10.7% and 10.3% lower for weeks 1, 7, 10, 11 and 13, respectively, when compared to control values. In addition, this reduction in feed consumption was dose-related for the entire 13 weeks of study (Table 11).

There were no statistical changes in feed consumption for female rats during this study; however, a minor but apparently dose-related decrease was noted (Table 12).

RDX intake (mg/kg/day) as computed from the actual body weight and feed consumption data is presented in Tables 13 and 14. The weekly adjustments of RDX concentration in the feed resulted in an overall dietary intake of RDX of less than 10% variation from designated dosage levels.

No unusual behavioral/pharmaco-toxic signs were noted in any animal during the course of this study. Except for one control female rat which accidently died during the 1 month tail-bleeding procedure, no mortality was observed during the course of this entire study.

2. Clinical pathology: Hematologic values for 30, 60 and 90 days of study are shown in Tables 15 to 20, and clinical chemistry data representing termination values are shown in Tables 21 and 22.

a. Hematology: At 30 and 60 days on test, male rats receiving 28 and 40 mg/kg/day of RDX showed a significant and dose-related decrease in hematocrit and hemoglobin values. After 90 days of RDX treatment, however, there was a pronounced increase (76%) in reticulocytes in this highest dose group male rats. This is suggestive of stimulation of the erythropoetic system by RDX. Other noteworthy changes in male rats were a statistically significant and dose-related increase in platelet count at the 3-month interval. Other changes in hematology values in male rats were variable, but minor dose-related trends were noted in erythrocyte count (decrease at 1 and 2 months), neutrophils (decrease at 1 month), and lymphocytes (increase at 1 month).

Female rats after 1 month feeding of RDX demonstrated changes in reticulocytes (79% increase for female rats at 28 mg/kg/day), minor statistical decreases in calculated ratios of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCHB) in the 28 mg/kg/day group and an elevated white blood count for female animals receiving 40 mg/kg/day. The reticulosis observed in female animals at the 28 mg/kg/day RDX group is accompanied by a nonstatistical increase in erythrocytes. This would account in part for the decreases in MCV and MCHB.

In contrast, after 2 months on test, the reticulocyte count rebounds and statistically decreases to 35% (highest dose group) of control animal value. After 3 months, however, no statistical changes are noted in reticulocyte count or other hematologic values (Table 15-20).

b. Clinical chemistry: Statistical decreases in glucose, SGPT, and serum potassium were recorded for male rats as well as in SGOT and serum sodium levels for female rats at termination of the 90-day study. All these values were within normal historical ranges.

3. Pathology:

a. Organ weights: Absolute and relative organ weights for rats fed RDX are found in Table 23. Noteworthy changes were seen as decreased heart weights in both male and female rats (40 mg/kg/day RDX groups). This was also reflected in heart weight relative to brain in both sexes. Other weight changes noted in spleen and gonads represent normal tissue variations.

b. Histopathology: Tissue lesions for control and the high dosage group in male and female rats are found in Tables 24 and 25. Tissue lesions were found with approximately equal frequency in both control and the treatment group (40 mg/kg/day), except as follows: pedunculated liver nodules in 1/10 male rats in the 40 mg/kg/day treatment group (probably an inborn anomaly); mild centrolobular cytoplasmic liver alterations in 1/10 treated male rats; mild liver granulomas in 1/10 treated rats; and an increased incidence of intestinal pinworm infestation in 8/10 treated male rats and 5/10 control animals. Pinworm infestation was also observed in 2/10 control female rats and 4/10 treated female rats. The significance of the increased incidence of pinworm in treated animals versus controls is not known at this time.

Also, female rats demonstrated a higher incidence of myocardial degeneration (minimum degree) in 2/10 control and 6/10 treated animals and a minimum degree of liver portal inflammation (1/10 for control versus 7/10 in treated animals).

C. Initial 90-Day Subchronic Study in Mice

1. Body weight, feed consumption, clinical signs and mortality: There were no meaningful changes in clinical signs, mean body weight gain or feed consumption data in either male or female mice throughout the entire 13-week period of RDX administration (Tables 26-29).

During week 10, one male mouse at the 28 mg/kg/day level was lethargic and considered moribund after losing 10 g body weight in a week. Necropsy found no apparent lesions. Because no similar effects were seen in other mice, including those fed higher doses, this death is deemed unrelated to the RDX feeding.

A nonspecific alopecia was noted in female mice during the study. This alopecia was believed to be attributed to cage mate interaction.

RDX intake (mg/kg/day) as computed from the actual body weight and feed consumption data is presented in Tables 30 and 31. The weekly adjustments of RDX feed mixture concentrations resulted in an overall dietary intake of RDX of less than 10% variation from designated dosage levels.

2. Clinical pathology: Hematologic and clinical chemistry determinations performed at termination are shown in Tables 32 and 33.

No meaningful changes were noted in hematology or blood chemistry values for mice administered RDX.

3. Pathology:

a. Organ weights: Absolute and relative organ weights for mice fed RDX are found in Table 34. No noteworthy changes were observed in these values.

b. Histopathology: Since there were no meaningful changes in mean body weight, feed consumption, hematology values, clinical chemistry values, terminal organ weights or pharmacotoxic signs, no histopathologic evaluation of these mice tissues was performed. Therefore, a supplemental study using RDX dosage levels of 80, 160 and 320 mg/kg/day was conducted to evaluate the maximum tolerated dose (MTD) for the chronic/carcinogenic study.

D. Supplemental 90-Day Subchronic Study in Mice

1. Body weight, feed consumption, clinical signs/mortality and RDX intake: Mean body weights of animals administered 320 mg/kg/day of RDX (highest dose group) in the diet were significantly higher than control values at week 10 for male mice (9.2%) and week 13 for female mice (5.8%). In addition, a slight but apparently dose-related increase in mean body weight was noted intermittently throughout the study in both sexes of mice (Tables 35 and 36). Since there were no change in mean feed consumption in test versus control animals (both male and female) throughout the entire 13-week period of RDX administration, the significance of the changes in body weight data is not known (Tables 37 and 38).

Hyperactivity and/or nervousness were noted in 50% of the male mice at weeks 7 and 8 of test; however, these signs were not seen in female mice during this study. Facial alopecia was observed in 3/12 female mice and 1/12 female mice at the 80 and 160 mg/kg/day dose groups, respectively;

however, the alopecia was not seen in any control or high dose group (320 mg/kg/day) animals or any male mice. This nonspecific alopecia in female mice has been seen in other studies, and is believed to be attributable to cage mate interaction.

Mortality was noted in 4/10 (40%) of the male mice and 2/12 (16.7%) of the female mice which received 320 mg/kg/day of RDX. All animals died during week 11 of test, except for one female mouse which died during week 6 (Table 39). No toxic signs were noted prior to death; however, as presented above, hyperactivity was noted during the 7th and 8th weeks of study in male mice.

RDX intake (mg/kg/day) as computed from the actual body weight and feed consumption data is presented in Tables 40 and 41.

The weekly and/or biweekly adjustments of RDX feed mixture concentrations resulted in an overall dietary intake of RDX of less than 10% overall variation from designated dosage levels:

Dosage Level RDX (mg/kg/day)	Percent (%) Variability from Nominal Dosage Levels	
	Male	Female
80	4.9	1.0
160	0.5	7.3
320	8.0	0.7

The following table summarizes changes related to body weight, pharmaco-toxic signs and mortality:

Parameter	Male Mice	Female Mice
Body Weight	9.2% ↑ in mean body weight gain in highest dose group at week 10.[a]	6.0% ↑ in mean body weight gain in highest dose group at week 13.[a]
Hyperactivity/ Nervousness	50% of male mice in 30 mg/kg dose group at weeks 7-8.	--
Facial Alopecia	--	Noted in 3/12 and 1/12 mice at 80 and 160 mg/kg/day groups, respectively. Not observed in control or highest dose group.
Mortality	4/10 (40%)[b] (high dose)	2/12 (16.7%)

a Significantly different from control group by Dunnett's multiple comparison procedure.

b Significantly different from control by Fisher's exact probability test.[2]

2. Clinical pathology: Results of the hematologic and clinical chemistry determinations performed at termination of study are found in Tables 42 (male) and 43 (female).

a. Hematology: Statistically significant changes when compared to control groups were noted as follows: (1) a 12% decrease in erythrocyte count in male mice receiving 160 mg/kg/day RDX, (2) a 7% decrease in hemoglobin concentration in males receiving 160 mg/kg/day, (3) an 8% increase in mean corpuscular volume (MCV) in male mice also receiving 160 mg/kg/day, and (4) a 6% and 5% increase in mean corpuscular hemoglobin (MCHB) in female mice receiving 80 and 160 mg/kg/day of RDX, respectively.

b. Clinical chemistry: SGPT values were decreased (30% to 47%) from control values in both male and female mice receiving 160 and 320 mg/kg/day RDX. The significance, if any, of decreases in SGPT values is not known.

Various hematologic/clinical chemistry result are tabulated as follows:

Parameter	Male Mice	Female Mice
RBC	12% ↓ in 160 mg/kg group[a]	--
Hb	7% ↓ in 160 mg/kg group[a]	--
MCHB	--	6% and 5% ↓ in 80 and 160 mg/kg group[a]
MCV	8% ↑ in 160 mg/kg group[a]	--
Platelets	↑	↑
Neutrophils	↑	↑
SGPT	↓	↓

a Significantly different from control by Dunnett's multiple comparison procedure.

↑/↓ Increase or decrease.

3. Pathology:

a. Organ weights: Absolute and relative organ weights for mice fed RDX are found in Table 44. A dose-related and significant increase in mean absolute liver organ weight and mean liver weights relative to body weight and brain weight was observed in both male and female mice. Also noted in male mice was a slight dose-related increase in mean absolute kidney weights and mean kidney weights relative to body weight.

Other noteworthy but minor changes were: (1) a dose-related increase in mean absolute brain weights and mean brain weights relative to body weight in male but not female mice, and (2) a dose-related decrease in mean gonad weights and mean gonad weights relative to body weights in male mice.

The following table summarizes the dose-related changes in organ weights:

Parameter	Male	Female
Liver weight	↑[a]	↑[a]
Liver (relative to body weight)	↑[a]	↑[a]
Liver (relative to brain weight)	↑[a]	↑[a]
Brain weight	↑	-
Brain (relative to body weight)	↑	-
Kidney weight	↑	-
Kidney (relative to body weight)	↑	-
Gonad weight	↓	-
Gonad (relative to body weight)	↓	-

a Highest dose group (320 mg/kg/day) significantly different from control group by Dunnett's multiple comparison procedure.

b. Histopathology: Tissue lesions for the control and the highest dose groups (320 mg/kg/day) of male and female mice are found in Tables 45 and 46. Tissue lesions were found with approximately equal frequency in both dose groups, except as follows: (1) a mild focal subscapular fibroplasia of the adrenal gland in 5/8 treated male mice versus 1/10 control animals; however, the significance of this finding in males is difficult to interpret since this adrenal lesion was present in all control and treated females; (2) a minimal to moderate focal myocardial degeneration in 5/9 treated male mice and a minimal degree in 2/11 treated female mice; (3) a mild to moderate periportal hepatocellular vacuolization in 5/9 treated male mice versus 3/10 control male mice--this lesion was not detected in any female mice; (4) an increased incidence of microgranulomas (mild degree) in 7/11 treated female mice versus 2/11 control female mice; (5) increased karyomegaly of hepatocytes (minimal) in 5/9 treated male mice but not female mice; (6) a mild tubulr nephosis in 4/9 treated male and 1/11 female mice, but not in any control animals; and (7) an increased incidence of dilated lumens (uterus) in 3/11 treated female mice versus 1/11 control animals.

The following table summarizes some of these findings:

	Male Control	Male 320 mg/kg Group	Female Control	Female 320 mg/kg Group
	Incidence of Lesion			
Adrenal - Focal subscapular fibroplasia (mild)	1/10	5/8[c]	11/11	11/11
Fat infiltration (mild)	-	-	7/11	10/11
Heart - Focal myocardial degeneration (minimal)	0/10	5/9[a,c]	0/11	2/11
Liver - Hepatocellular vacuolization - periportal	3/10	5/9[b]	-	-
Microgranuloma (mild)	2/10	1/9	2/11	7/11[c]
Increased karyomegaly of hepatocytes (minimal)	0/10	5/9[c]	-	-
Kidney - Tubular nephrosis (mild)	0/10	4/9[c]	0/11	1/11
Uterus - Dilated lumen	-	-	1/11	3/11

a One tissue showed a moderate lesion.
b Three tissues showed moderate lesions.
c Significantly different from control by Fisher's exact probability test.[2]

Histopathologic evaluation of male and female animals at the 320 mg/kg/day RDX groups which died before scheduled termination of study are summarized below:

Lesion	Lesion Incidence[a] Male	Female
Myocardial degeneration	1/3	-
Liver lesions (hepatocellular vacuolization)	2/3	-
Tubular nephrosis	3/3	1/1

a As a result of post mortem autolysis, one male and one female could not be evaluated histopathologically.

Liver lesions and degenerative changes in the myocardium are found in treated animals which died during the course of the study and in animals which were sacrificed after 90 days of test. This suggests that the injury or insult to the liver or heart cells may have taken place prior to the death or sacrifice of these animals.

IV. MUTAGENICITY STUDIES

A. Ames Salmonella/Microsome Test

The results of the mutagenicity tests on RDX are summarized in Table 47. The mutagenic index (defined as the ratio of the number of revertants on the test plate to the number of revertants on the corresponding control plate) of all five strains of *Salmonella typhimurium* to RDX was less than 2.0. The number of revertants produced by treatment of the cultures with RDX at all concentrations was approximately the same as the vehicle-treated controls. The positive controls were all mutagenic in their appropriate tester strains, indicating that the metabolic activation system was working properly and all strains were capable of mutation.

B. Dominant Lethal Mutation Study in Rats

1. Body weight and feed consumption: The males were fed RDX at nominal doses of 0, 5, 16 or 50 mg/kg/day for 15 weeks before this study began. Males of the high dose group ate less and gained less weight than controls, as discussed more fully below under the two-generation reproduction study, and in Tables 60, 65 and 69.

2. Mating: The number of male rats that were tested for possible dominant lethal effects resulting from consuming RDX-containing diets is presented in Table 48. In addition, this table indicates the number of tested males that impregnated various numbers of untreated females. All of the mid and high dose RDX-treated males were judged to be fertile on the basis of their ability to impregnate at least one female. One male in the low dose group was judged to be infertile; however, this was not considered to be significant, even though infertility was not observed in other groups, as there was no dose-related effect. Although the pregnancy rate appeared lower in females mated with males that received the high dose, this may be related to adverse effects of treatment on the general well-being of these males.

Pregnancies which resulted from the 2-week mating period were normal. There was no statistically significant effect on the number of corpora lutea implants, live embryos, or dead embryos during either the first week (Table 49) or second week (Table 50) of mating. In addition, when the results of the first and second week of mating were combined (Table 51), there was no statistically significant change in any of the above parameters.

V. REPRODUCTIVE TOXICITY STUDIES

A. Teratology Study in Rats

1. Dose range finding study: Dose range finding was carried out in pregnant rats given RDX suspensions via oral route at 10, 20, 40 and 80 mg/kg/day. All rats given an RDX dose of 80 or 40 mg/kg/day died following convulsions. Inspection of the uterus at the time of death indicated that all dams in the 40 mg/kg/day but not in the 80 mg/kg/day dose group had hemorrhages around implantation sites or detached feto-placental units. Similar effects were not observed at lower doses with full-term dams. With the exception of one dam given 20 mg/kg/day which exhibited some of the jerking movements, no effects were seen in dams given 10 or 20 mg/kg/day when compared with dams given the suspension medium without RDX. An indication of morbidity was evident in the weight gain of these dams. Mean values for the treatment weight change (difference between gravid day 20 and day 6 body weight) were 91 or 86 g for dams given 10 or 20 mg/kg/day versus 126 g for the control. No indications of an adverse effect on fetal development was observed. This range finding teratology study in rats suggests that at dosage levels of 20 mg/kg/day RDX a consistent adverse effect on maternal weight gain and intrauterine effects may be observed. As a result of this study, three dose levels of RDX (i.e., 0.2, 2, and 20 mg/kg/day) were selected for the full-scale study.

2. Maternal toxicity: The effects of RDX or hydroxyurea treatment on pregnant rats are presented in Table 52.

a. Body weight: RDX at a dose of 20 mg/kg/day caused a decrease in maternal body weight. The corrected body weight of dams given 20 mg/kg/day was significantly reduced. This parameter provides a measure of effects of RDX on the dam which are independent of effects on litter size and is obtained by subtracting the day 0 body weight and the weight of the uterus plus contents from the day 20 body weight. This reduction in corrected body weight gain was the result of losses in body weight which occurred following the first three doses of RDX, that is, from gestational day 6 to day 9. The weight gain for gestation days 9-13 was not significantly reduced. The significantly reduced final body weight indicated that neither additional losses nor compensation for initial losses occurred during the remainder of gestation. No effects on body weights were observed with dams treated with either lower dosages of RDX or hydroxyurea.

b. Feed consumption: During the gestation days 6-9 (i.e., following three doses of RDX) a significant reduction in feed consumption was noted. In the rats treated with RDX at 20 mg/kg/day dosage level a gradual recovery of feed consumption was observed during days 9-13. A near complete recovery of feed intake was reached on day 19 of the gestation period. There were no changes in feed consumption noted in any other treated groups.

c. Mortality: Mortality was observed in 6 of 25 dams treated with 20 mg/kg/day of RDX (Table 52). Two deaths occurred on the 11th, three on the 12th, and one on the 14th day of gestation, after five, six, or eight doses of RDX, respectively. Chronic convulsions were observed in one of the dams before death. Similarities in the appearance of the dam that died (e.g., dried blood around the mouth and the nose and bloody stain in the cage) following convulsions suggested that convulsions occurred in all four dams before death. One dam was accidentally killed during the course of the study. No death occurred in the other treatment groups.

d. Neurotoxicity: Neurotoxic signs of RDX reported previously were observed in 18 of the 25 dams treated with 20 mg/kg/day. These animals showed hyperactivity and other central nervous system related stimulations including convulsions. The appearance of neurotoxic signs in these animals normally occurred on the second day of dosing and then diminished in frequency after the eighth day of dosing. At a dose level of 2.0 mg/kg/day, only one female exhibited convulsion during the period of dosing. Convulsions were also observed in one female receiving 350 mg/kg/day of hydroxyurea. There were no neurotoxic signs observed in any females receiving either the vehicle or 0.2 mg RDX/kg/day during the dosing period of days 6-19 of gestation.

e. Maternal liver weights: The absolute liver weight was significantly reduced in dams treated with 20 mg/kg/day of RDX. There were no meaningful changes in liver weight in dams treated with low dose levels of RDX or hydroxyurea.

f. Reproductive parameter: The effects of RDX on maternal reproduction are presented in Table 52. There were no meaningful changes in number of implants, visible, and dead fetuses when the data obtained from treated animals were compared with control animals. High incidence of earlier resorption was noted in surviving animals treated with RDX at 20 mg/kg/day. However, it is difficult to evalute the significance of this parameter based on only surviving dams.

3. Fetal anomalies: The effects of RDX or hydroxyurea treatment on fetuses are summarized in Tables 53-55. The highest dose level of RDX at 20 mg/kg/day is embryotoxic to rats.

a. Gross anomalies: The external anomalies detected in fetuses from rats following oral administration of RDX at 0.2, 2.0, and 20.0 mg/kg/day were found to be similar to control rats receiving vehicle only. Hydroxyurea, serving as positive control, produced high incidences of cleft palate, abnormal snout, absence of eye bulges, etc.

b. Soft tissue anomalies: The soft tissue anomalies detected in fetuses from rats following various oral doses of RDX were similar to those rats receiving vehicle only. However, incidence of hydrocephalas and micropthalmia were found to be statistically higher in hydroxyurea-treated rats as compared with control rats.

c. Skeletal anomalies: There were no meaningful skeletal anomalies found in RDX-treated animals at the highest dosage level of 20 mg/kg/day. The frequency of observed skeletal anomalies in RDX-treated animals was similar to those rats receiving vehicles only. Hydroxyurea, a positive teratogen, produced significantly higher incidences of various anomalies of snout, mandible, cranium, vertebrae, sternebrae, axial skeleton and ribs in rats than in either the vehicle- or RDX-treated rats.

B. Teratology Study in Rabbits

1. Maternal welfare and reproduction: The effects of RDX and 6-aminonicotinamide on maternal welfare and reproduction are presented in Table 56. A statistical comparison of maternal body weights at weekly intervals during gestation indicated that none of the experimental groups was significantly different from the control group. However, a visual inspection of the data suggested that dams who received 6-aminonicotinamide and 20 mg/kg/day of RDX gained less weight during gestation than controls. Neither of these treatments affected the number of implants per dam. In addition, treatment with RDX did not affect the percent of viable or dead fetuses and the incidence of early and late resorptions. In contrast, 6-aminonicotinamide increased the incidence of early resorptions. The number of fetuses per dam and fetal weight were normal in all groups treated with RDX. In contrast, the number of fetuses per dam was reduced in the group that received 6-aminonicotinamide. In addition, the fetal weight in this group appeared to be reduced.

2. Gross anomalies: The gross anomalies observed in pups from dams that received 6-aminonicotinamide or RDX are presented in Table 57. The great majority of anomalies were observed in the group that received 6-aminonicotinamide. In this group, anomalies such as reduced eye bulges and cleft palate occurred in 68 and 35% of the fetuses, respectively, and 88 and 62% of the litters, respectively. A broad spectrum of other anomalies was also observed in this group. In contrast, groups that received 0.2 and 2.0 mg/kg/day of RDX were free of gross anomalies other than small fetuses, which were also observed in the control group. Pups from the group that received 20 mg/kg/day of RDX appeared to have more anomalies than did the other RDX-treated groups. For example, the incidence of spina bifida, which was not observed in the control or other RDX-treated groups, was 3% based on the number of fetuses and 18% based on the number of litters. Other anomalies which were observed in the group treated with 20 mg/kg/day of RDX and not in the control or other RDX-treated groups included: misshaped cranium, meningocele, misshaped eye bulges, enlarged eye bulges, abdominal wall defects, gastroschisis, appendicular reduction anomalies, and problems with the tail. The incidence of these anomalies ranged from 1 to 3% of the fetuses in 9 to 18% of the litters; none of these increases were statistically greater than the control values.

3. Soft tissue anomalies: Soft tissue anomalies observed in pups from dams that received 6-aminonicotinamide or RDX are presented in Table 58. As previously reported, 6-aminonicotinamide produced a broad spectrum of anomalies with an incidence that varied from one fetus to many fetuses.

Anomalies were also observed in pups from dams that received RDX; however, these anomalies tended to occur also in the control group (e.g., trachea occluded and small fetus) or only in the low and or mid dose groups (e.g., nasal passage occluded, stomach distended, and urinary bladder distended). Cleft palate was also observed in RDX-treated groups; however, the incidence did not increase in a dose-related manner and, as with other anomalies in the RDX-treated groups, the increase was not statistically significant.

4. Skeletal anomalies: Skeletal anomalies observed in pups from dams that received 6-aminonicotinamide or RDX are presented in Table 59. As previously observed, 6-aminonicotinamide produced a broad spectrum of anomalies with an incidence that varied from one to many fetuses. In general, anomalies observed in RDX-treated groups occurred with a low incidence and did not increase in a dose-related manner. Enlarged frontal fontanel occurred only in the chemical-treated groups, and the incidence in RDX-treated groups ranged from 4 to 15% of the fetuses in 17 to 18% of the litters; however, this was not a dose-related increase.

C. Two-Generation Reproduction Study

1. Actual doses of RDX consumed by rats: The concentration of RDX in the diet was varied at weekly intervals in an effort to provide a constant daily dose of the test material as the rats increased in weight. The diets were prepared to provide groups of male and female rats with 5, 16, and 50 mg/kg/day of RDX. The actual doses of RDX consumed during the study by F_0 males (Table 60), F_0 females (Table 61), F_1 males (Table 62), and F_1 females (Table 63) are presented in the indicated tables. The treatment groups are identified in these and subsequent tables either as low, middle, or high dose groups or as receiving nominal RDX doses of 5, 16, and 50 mg/kg/day.

2. General health and well-being of rats that consumed diets containing RDX

a. Survival: The total number of male and female rats during various portions of this study is presented in Table 64. Survival was not affected in the low and mid-dose groups. In contrast, mortality in the high dose group was 18% during the F_0 generation. In addition, this group had the highest percentage of stillborn pups during the F_1 and F_2 generations with 17 and 52%, respectively.

b. Body weights: The body weights of F_0 males (Table 65), F_0 females (Table 66), F_1 males (Table 67), and F_1 females (Table 68) during the study are presented in the indicated tables. In general, the body weights of both males and females in the high dose group were consistently reduced. During a few of the observations of the low and mid-dose groups, body weights of the F_1 generation were also reduced. However, after test week 22 there was no significant effect on body weight in either males or females in the low dose group. Likewise, after the 27th week there was no significant effect on body weight of either males or females in the mid-dose group. In summary, a consistent reduction in body weight of both males and females during the F_0 and F_1 generations was observed only in rats from the high dose group.

c. Feed consumption: The feed consumption of F_0 males (Table 69), F_0 females (Table 70), F_1 males (Table 71), and F_1 females (Table 72) is presented in the indicated tables. These values were consistently reduced during the F_0 and F_1 generations in both males and females from the high dose group. Intermittent reductions were also observed during the F_1 generation in males and females from both the low and mid-dose groups; however, these effects were not long-lasting.

3. Reproduction

a. Results of first F_0 mating: The numbers of adults, litters, and pups during this mating are presented in Table 73. In addition, the gestational body weight of dams and lactational body weight of their pups are presented in Table 74. The numbers of males that mated (i.e., produced sperm-positive vaginal smears in females with which they were cohoused) and were judged to be fertile (i.e., fathered litters with at least one viable pup) were reduced in the high dose group; however, the magnitude of this change did not reach a level of statistical significance. These parameters were normal in the low and mid-dose groups. Adverse effects on reproduction similar to these effects observed in males from the high dose groups were seen in the females with which they were cohoused. In other words, the numbers of females that mated (i.e., had sperm-positive vaginal smears) and were pregnant (i.e., produced litters with at least one viable pup) were reduced in the high dose group; however, this effect, too, was not statistically significant. In addition, the gestational body weight of dams in the high dose group was reduced.

The viability of pups, as monitored by the number of litters with at least one viable pup and the number of pups per litter, was reduced in the high dose group. These parameters were not adversely affected in the low and mid-dose grups. The body weight of pups in both the mid- and high dose groups was reduced 25 days after birth.

b. Results of second F_0 mating: In the second mating of the F_0 generation, females from the control and high dose groups were mated with nontreated proven male breeders. The results of these matings are presented in Table 75. Although 80% of the females in both groups mated, the pregnancy rate in both groups was 20%. Since the pregnancy rate was low in both the control and treated groups, it is not possible on the basis of these data to attribute these observations to RDX. Although the number of litters is low in both groups, the data do suggest that the high dose of RDX adversely affects pup survival.

c. Results of F_1 mating: The numbers of adults, litters, and pups during this mating are presented in Table 76. In addition, the gestational body weight of dams and lactational body weight of their pups are presented in Table 77. These observations do not permit meaningful conclusions to be made concerning treatment-related effects in the high dose group because the four males and two females which were available for mating came from the same litter; as a result, the number of rats mated was small. Nevertheless, the data do suggest that treatment with the high dose adversely affected pup survival. The gestational body weight of dams in both the mid- and high dose groups was significantly reduced.

d. Summary of observations on reproduction: The above observations are summarized in terms of various indexes in Table 78. In the high dose group the fertility, viability, and lactation indexes were reduced.

4. Histopathological evaluation: This portion of the report deals with the morphologic, gross, and microscopic study of weanlings of the F_2 generation sacrificed on day 21. The data which were available for evaluation consisted of gross observations at autopsy, body weights and the weights of six organs (brain, heart, liver, kidney, spleen and gonads), and microscopic findings on about 30 different tissues from 10 males and 10 females in each dose group.

Gross observations at the time of necropsy revealed no differences between the experimental groups and the controls.

Comparison of the body weights indicated that both the males and females from the mi -dose group (16 mg/kg/day) weighed less than the controls (Table 79). In addition, the absolute weights of male gonads and female kidneys and spleen were significantly reduced. Histopathological examination of the tissues, as described below, did not provde an explanation for these differences.

Histopathological examination disclosed an increased number of renal tubular epithelial-lined cysts in the cortex of the kidneys of the mid-dose group. Similar cysts were present in both control and low dose groups (Table 80). The cysts occurred bilaterally mainly in the outer cortex; they were multiple, congenital, benign, and were not associated with renal interstitial inflammatory cell infiltrates, nor with tubular casts or evidence of tubular cell degeneration. They were not caused by intratubular obstruction. They most likely resulted from dysgenesis of nephron formation. The increased number of cysts in the mid-dose animals suggested an RDX-related effect. Renal cystic disease has been induced in adult rats by various compounds. Induction in utero of cystic tubular lesions has been achieved by feeding dams commercially aged diphenylamine containing DPA-derived impurities.[12]

Histologic study of the thymus revealed subtle changes in two and a distinct change in a single female of the mid-dose group. While the thymus gland of all control animals was remarkably similar and uniformly nomal, the experimental groups showed a slightly greater degree of variablity which was considered within the range of normal. The change consisted of a relative diminution of small thymocytes (lymphocytes) in the thymic medulla, exposing the epithelial reticulum and prominent Hassall's corpuscles. A single occurrence of epithelial-lined cyst formation was noted. This was accompanied focally by a severe cytophagocytosis of lymphocytes. Spontaneous occurrence of similar structures in the rat thymus is not discussed in the literature consulted[13,14,15] but has been previously observed.[16] They have been produced in both male and female rats by chronic estrogen administration.[16] The changes noted differ from those observed in acute involution of the thymus as part of the stress reaction. The thymic changes observed were not associated with any alteration of the spleen and lymph nodes. Comparison of control and experimental groups revealed no difference with regard to the latter organs. The thymic changes were not considered significant. Toxicologically they may possibly be related to RDX treatments, but this cannot be proved.

VI. DISCUSSION

A. Toxicity Studies

1. Acute oral toxicity: Previous acute oral toxicity (LD_{50}) studies performed with RDX in white rats (von Oettingen et al.[7]) and white mice (Sklyanskaya and Pozharlskiy[8]) have reported LD_{50} values of 145 and 377 mg/kg, respectively. The primary adverse effect of RDX in these studies was on the central nervous system as evidenced by hyperreflexia, shivering, piloerection, Straub tail and, at high doses, convulsions. The Straub tail phenomenon is suggestive of spinal cord involvement.

The acute toxicological responses and the LD_{50} values here noted in response to RDX in both species of animals suggests both qualitative and quantitative similarities in rodent species. In fact, comparison of the actual LD_{50} values (118 mg/kg for rats versus 80 mg/kg for mice) does suggest that RDX is equally toxic to both rats and mice; however, the very steep dose-response curve (slope = 10 3) for rats (combined sexes) and the relatively flat dose-response curve (slope = 2.3) for mice (combined sexes) is indicative of a delayed RDX toxicity. Concerning the mode of death in these animals, the convulsive episodes/gasping symptoms is consistent with death by anoxia.

The LD_{50} in rats reported by von Oettingen et al.[7] is consistent with the LD_{50} value reported in this study (LD_{50} in Fischer 344 rats = 118 mg/kg); however, the LD_{50} in mice reported by Sklyanskaya and Pozharlskiy[8] is four to five times higher than the LD_{50} reported on B6C3F1 mice in this study (LD_{50} in B6C3F1 mice = 80 mg/kg). The difference noted in mice may be due to the strain difference and/or the suspending vehicles: Sklyanskaya and Pozharlskiy used linseed oil while this study utilized methyl cellulose-polysorbate 80 combination.

McNamara et al.[17] found that the intravenous LD_{50} of RDX in mice was 18.7 mg/kg, about one-fourth of our oral value. Toxic signs (convulsions, labored breathing, lethargy) were similar to those we saw, so the difference probably reflects absorption differences, rather than toxic mechanism differences.

B. Subchronic Toxicity

Previous 90-day subchonic studies performed with rats revealed one of the 20 animals fed 15 mg/kg/day died during the second week of exposure; 8 of 20 animals in the 25 mg/kg/day group and 8 of 20 animals in the 50 mg/kg/day group died (von Oettingen et al.[7]). Toxicological signs included convulsions, hyperirritability and viciousness in the 25 and 50 mg/kg/day group. Congestion of the lungs and gastrointestinal tract was observed in animals which died during the course of the study.

These subchronic studies are intended to identify doses for subsequent chronic/carcinogenic studies so the discussion is cast along those lines.

a. Rats: The most significant effect seen in the 90-day study of rats was the dose-related changes in body weight gains in both sexes. Since the primary purpose of this subchronic study was to determine the maximum tolerated dose (MTD) for the future combined chronic and carcinogenic study, extrapolation of the body weight dose-response curves yields an approximate MTD equal to 50 mg/kg/day of RDX for the rat. Additional information for recommending this MTD are: (1) a slight decrease in hemoglobin, hematocrit, and glucose levels observed in the 40 mg/kg/dy male rat groups during the study; (2) an apparent dose-related reduction in absolute and relative (to body weight and brain weight) terminal heart weights in both male and female rats; (3) a dose-related decrease in absolute spleen weights in female rats; (4) a decrease in relative spleen weights (relative to brain) compared to control vaues in female rats receiving 40 mg/kg/day of RDX; and (5) an increased incidence of foci of myocardial degeneration in female rats (40 mg/kg/day group) versus control animals.

The no effect, or low dosage level for a rat chronic/carcinogenic study, is recommended at 15 mg/kg/day, since RDX at 14 mg/kg/ day did not have any toxicologically important effect on any parameters measured in the 90-day subchronic study. We recommend that the mid-high dosage level be set at 75 mg/kg/dy which would depress the body weight gain in female rats, further depress the body weight gain in male rats, and produce changes in hematologic values. For this level would no doubt produce mortality in rats and allow better characterization of target organ toxicity.

b. Mice: The initial subchronic mouse study found no effects in mice fed the same doses as the rats. Therefore, as reported above, the study was repeated at higher doses.

The maximum tolerated dose (MTD) for B6C3F1 mice for a carcinogenic study was not established from this supplemental 90-day study since no meaningful changes were noted in mean body weight gains in RDX treated animals versus control animals. However, since mortality was noted in both male and female mice receiving RDX at 320 mg/kg/day, the MTD would be predicted to be less than this dosage.

Intermittent changes in hematology parameters (RBC, Hb, MCV, platelets, neutrophils, and eosinophils in both sexes) suggest an involvement of RDX on the erythropoietic system.

A dose-related increase in absolute mean liver organ weights (also liver weights relative to body weight and brain weight) was evident in both male and femae mice. The increase in mean liver weights is consistent with the findings of French et al.,[9] who showed by electron microscopy that RDX induced the proliferation of the smooth endoplasmic reticulum (SER) of the liver. The increased incidence of periportal hepatocellular vacuolization observed in treated male mice (320 mg/kg/day group) during this study is consistent with the above findings and suggests that the liver is, in part, the site of RDX metabolism.[10]

Other noteworthy organ weight changes observed in male but not female mice were dose-related increases in mean brain and kidney weights and a dose-related decrease in mean testes (gonad) weight. The significance of the increased brain and decreased testes weight is not known. The increased mean brain weight (edema?) might be the basis of the hyperirritability noted in the highest dosage group male mice during weeks 7 through 8 of this study; however, the hyperirritability was not noted after week 8. Kidney weight changes in male mice correspond somewhat to the histopathological findings of renal tubular nephrosis in animals which died during the study. (Future animal studies with RDX might be designed to measure urine protein as a potential qualitative indicator of RDX toxicity.)

Focal myocardial degeneration was evaluated in treated male (5/9) and female (2/11) mice but was not noted in control animals. The significance of this lesion in mice (highest dose group) is not known.

C. Mutagenicity Studies

1. Ames *Salmonella*/microsome test: In all strains of *S. typhimurium* used, RDX produced approximately the same number of revertants as the vehicle-tested controls. The simultaneous positive controls produced the expected mutagenic effects. Therefore, RDX is not mutagenic in the Ames *Salmonella*/microsome test, under the conditions of this study.

2. Dominant lethal mutation study in rats: Again, under the conditions of this test, there was no indication of any dominant lethal mutation effect (decreased implants and/or increased dead and nonviable embryos) in the rats.

D. Reproductive Study

1. Teratology studies: In order to be classified as a teratogen by the usual definition, an agent must alter the structure or function of a statistically significant number of young.[11] An agent is not classified as a teratogen if it only produces fetal death or reduces fetal growth. In addition, an agent is not classified as a teratogen if the dose required to produce an effect in the embryo or fetuses is overtly toxic to the dam.

In rats, the high dose (20 mg/kg/day) of RDX produced maternal toxicity (neurotoxicity, including convulsions), some maternal deaths and embryotoxicity. The embryotoxicity could be solely the result of the convulsions and consequent hypoxia/transient anoxia. The lower doses (0.2 and 2.0 mg/kg/day) had no adverse effects. In rabbits, maternal well-being, as monitored by body weight, was not adversely affected in dams that received 0.2 or 2.0 mg/kg/day of RDX. The weight gain of dams that received 20 mg/kg/day or RDX was slightly reduced.

In both species, no specifically teratologic effects were observed in RDX-treated animals. The positive controls did produce the expected teratologic effects showing the responsiveness of the test system.

2. Two-generation reproduction study: Evidence of toxicity was observed in adult rats from the group that received the high dose (50 mg/kg/day) of RDX. These effects included death, reduced body weight, and reduced feed consumption. Although some of these parameters occasionally were affected in the low (5 mg/kg/day) and mid-dose (16 mg/kg/day) groups, the effect was not long-lasting. Therefore, it is concluded that these parameters of toxicity were affected primarily in the high dose group.

Reproductive performance was affected in males and females from the high dose group. These effects included a reduced number of pregnancies and a poor survival of the offspring from the pregnancies. Although the reduction in pregnancies did not reach a level of statistical significance, the effects on survival were dramatic. Two observations suggest that the source of this effect may reside in the female. First, as reported above, matings between treated females and non-treated males produced similar effects. However, the strength of this observation was weakened both by the small number of pregnancies and the poor mating performance of control animals. Second, as reported in the dominant lethal mutation study, matings between treated males and nontreated females produced pregnancies which were normal during a midgestational examination. However, this study did not evaluate the survival of the offspring. In contrast, the reproductive performance of rats in both the low and mid-dose groups was essentially normal.

In terms of the histopathological evaluation of the F_2 generation, two RDX-related findings were of statistical significance: an increase in renal cortical cysts and reduced body weights of mid-dose females, but not of males.

VII. CONCLUSIONS

A. Toxicity

1. Acute oral LD_{50}s ± standard error in rats were 119.0 ± 4.6, 118.7 ± 4.5 and 118.1 ± 2.8 mg/kg for males, females and combined sexes. The analogous LD_{50}s for mice were 97.2 ± 8.7, 58.9 ± 26.8, and 80.3 ± 9.6 mg/kg, respectively. Sex differences were not statistically significant.

2. Toxic signs were those of neurotoxicity: gasping, labored breathing, clonic/tonic convulsions. Convulsions could be induced in mice by a finger-snap; some had transient Straub tail.

3. In a 90-day subchronic feeding study, a dose of 40 mg/kg/day was toxic to rats, decreasing weight gain and, in males, feed consumption in some weeks. More specific toxic effects (such as decreased hematocrit and SGPT) were small, inconsistent and of little toxicological importance. Feeding 28 mg/kg/day produced no apparent toxic effects.

4. In 90-day subchronic feeding studies, RDX was less toxic to mice than to rats. Effects seen in mice fed 40 mg/kg/day for 2 weeks and 320 mg/kg/day for 11 weeks include hyperactivity in males, unscheduled deaths (especially in males), increased liver size accompanied by periportal hepto-cellular vacuolization (males) or microgranulomas (females) and increased kidney weight with mild tubular nephrosis in male mice. No toxicologically important effects were observed in mice fed lower doses. The maximum tolerated dose is < 320 mg/kg/day (but > 40 mg/kg/day); in the absence of histo-pathological examination of lower doses, no more precise estimate can be made.

B. Mutagenicity Studies

1. RDX was not mutagenic in the Ames *Salmonella*/microsome test at doses up to 1 mg/plate.

2. RDX was not mutagenic on the rat dominant lethal mutation test at doses up to 50 mg/kg/day.

C. Reproductive Toxicity

1. RDX was not teratogenic to rats, although considerable maternal toxicity (primarily neurotoxicity) and lethality and embryotoxicity were induced at doses of 20 mg/kg/day. No effects were seen at doses of 2 mg/kg/day.

2. RDX was not teratogenic to rabbits at doses up to 20 mg/kg/day. The rabbits were less sensitive to the general toxicity of RDX than the rats.

3. In a rat two-generation reproduction study, doses up to 50 mg/kg/day had considerable general toxicity (particularly neurotoxic effects and including unscheduled deaths), but no specific reproductive effects other than those which could be ascribed to poor nutrition from the general toxicity. Lower doses (5 or 16 mg/kg/day) produced no apparent toxicity.

LITERATURE CITED

1. Finney, D. J., Statistical Methods in Biological Assay, Griffin, London, 1971.

2. Steel, R. G. D., and J. H. Torrie, Principles and Procedures of Statistics, McGraw-Hill Book Co., New York, pp. 109-110, 1960.

3. Dunnett, C. S., A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J. Amer. Statist. Assoc., 50:1096-1121, 1955.

4. Wilson, J. G., Methods for Administering Agents and Detecting Malformations in Experimental Animals, in Teratology--Principles and Techniques, J. G. Wilson and J. Workany (eds.), University of Chicago Press, Chicago, Illinois, pp. 262-277, 1965.

5. Staples, R. C., and V. L. Schnell, Refinements in Rapid Clearing Techniques in the KOH-Alizarin Red S Method for Fetal Bones, Stain Technol., 39:61-63, 1964.

6. Mann, H. B., and D. R. Whitney, On a Test of Whether ne of Two Random Variables is Stochastically Larger than the Other, Ann. Math. Stat., 18:50-60, 1947.

7. von Oettingen, W. F., D. D. Donahue, H. Yagoda, A. R. Monaco, and M. R. Harris, Toxicity and Potential Dangers of Cyclotrimethylenetrinitramine (RDX), J. Ind. Hyg. Tox., 31, 21-31, 1949.

8. Skylanskaya, R. M., and F. I. Pozharlskiy, Questions on the Toxicity of Hexogen, Farm. i Toks., 7, 43-47, 1944.

9. French, J. D., S. L. Bradley, N. R. Schneider, M. E. Andersen, and L. J. Jenkins, "Cyclotrimethylene-trinitramine (RDX) - induced Ultrastructural Changes in Rat Liver and Kidney, Toxicol. Appl. Pharmacol., 37, 122, 1976.

10. Schneider, M. R., S. L. Bradley, and M. E. Andersen, Metabolism of [^{14}C] Cyclotrimethylene Trinitramine (RDX) in the Rat, Toxicol. Appl. Pharmacol., 37, 137, 1976.

11. Staples, R. E., and J. G. Wilson, Definition of Teratogenesis and Teratogen, in Methods for Detection of Environmental Agents that Produce Congenital Defects, T. H. Shepard and J. R. Millers (eds.), American Elsevier Publishing Company, New York, pp. 25-26, 1975.

12. Crocker, J. F., D. M. Brown, R. F. Borch, and R. L. Vernier, "Renal Cystic Disease Induced in Newborn Rats by Diphenylamine Derivatives," Am. J. Pathol., 66:343-350, 1972.

13. Andreasen, E., "Studies on the Thymolymphatic System: Quantitative Investigation on the Thymolymphatic System in Normal Rats at Different Ages, Under Normal Conditions and During Inanition and Restitution After Starvation," Acta-Path. Microbiol. Scand., Supplement 49, 1943.

14. van Haelst, U., "Light and Electron Microscopic Study of the Normal and Pathological Thymus of the Rat: I. The Normal Thymus," Ztschrft Zellforschung u. Mikrosk. Anat., 77:534-553, 1967.

15. van Haelst, U., "Light and Electron Microscopic Study of the Normal and Pathological Thymus of the Rat: II. The Acute Thymic Involution," Ztschrft Zellforschung u. Mikrosk. Anat., 80:153-182, 1967.

16. Linhartova, A., "Experimentelle Zystose des Thymus," Zentralblatt F. Allg. Pathol. u. Pathol. Anat., 105:340-349, 1964.

17. McNamara, B. P., H. P. Averill, E. J. Owens, J. F. Callahan, D. G. Fairchild, H. P. Cinchta, R. H. Rengstorff, and D. K. Biskup, "The Toxicology of Cyclotrimethylenetrinitramine (RDX) and Cyclotetramethylenetetranitramine (HMX) Solutions in Dimethylsulfoxide (DMSO), Cyclohexanone, and Acetone," Technical Report EB-TR-73040, April 1974, NTIS Report No. AD-780,010.

TABLE 1

ACUTE ORAL TOXICITY (LD_{50}) OF RDX IN RATS

Dose (mg/kg)	Mortality No. Dead/No. Dosed Male	Female	Total (%)	Number of Deaths 1-6 hr	7-24 hr	1-14 days
250[a/]	10/10	10/10	20/20 (100)	20		
200[a/]	9/10	10/10	19/20 (95)	19		
180[a/]	10/10	10/10	20/20 (100)	20		
150[a/]	10/10	10/10	20/20 (100)	20		
125[b/]	8/10	6/10	14/20 (70)	3	11	
100[c/]	0/10	1/10	1/20 (5)		1	

	LD_{50} ± S.E.	95% Confidence Limits	Slope ± S.E.
MALE:	119.0 ± 4.6	110.4 - 128.3	17.25 ± 11.49
FEMALE:	118.7 ± 4.5	108.0 - 128.9	8.36 ± 2.38
COMBINED:	118.1 ± 2.8	111.8 - 124.1	10.32 ± 2.20

TOXIC SIGNS:

a/ Gasping and/or labored breathing, clonic/tonic convulsion, and death within 3 hr of RDX administration. One male rat dosed with 200 mg/kg RDX exhibited these toxic signs but survived to termination of study.

b/ 14/20 animals dosed at 125 mg/kg RDX exhibited gasping/labored breathing, and clonic/tonic convulsions. Of these 14 animals, 3 died within 3 hr (2 males and 1 female). Eleven animals were found dead the next morning (death between 6-22 hr after dosing). One male convulsed but survived to termination of study. The surviving 5 rats did not exhibit any toxic signs.

c/ One male rat was observed convulsing 4 hr after RDX administration but survived to termination. The one female rat, which died at 6-22 hr of RDX administration and the remainder of surviving animals did not exhibit toxic signs.

TABLE 2

ACUTE ORAL TOXICITY (LD_{50}) OF RDX IN MICE

Dose (mg/kg)	Mortality No. Dead/No. Dosed Male	Female	Total (%)	Number of Deaths 1-6 hr	7-24 hr	1-14 days
350[a]	5/5	5/5	10/10 (100)	10		
225[a]	5/5	5/5	10/10 (100)	10		
180[a]	5/5	5/5	10/10 (100)	10		
140[b]	5/5	4/5	9/10 (90)	9		
100[b]	3/5	3/5	6/10 (60)	6		
60[c]	0/5	3/5	3/10 (30)			3

	LD_{50} ± S.E.	95% Confidence Limits	Slope ± S.E.
MALE:	97.2 ± 8.7	81.6 - 115.8	8.87 ± 17.53
FEMALE:	58.9 ± 26.8	24.8 - 139.5	1.20 ± 0.78
COMBINED:	80.3 ± 9.6	55.3 - 99.2	2.31 ± 0.65

TOXIC SIGNS:

a/ All animals at 350, 225, and 180 mg/kg RDX were observed to have severely labored-intense gasping, violent clonic/tonic convulsions, and death within 5-10 min after RDX administeration. Convulsions could also be induced by a finger snap. Mice at 350 mg/kg RDX also exhibited transient Straub tail-like symptoms.

b/ All animals which died at 100 and 140 mg/kg RDX displayed clonic/tonic convulsions and died within 30 min after test compound administeration. Convulsions could also be induced by a finger snap (loud noise).

c/ No toxic signs were noted prior to death in the three female mice which were found dead after 10 days on test.

TABLE 3

PROBIT ANALYSIS OF MORTALITY DATA FOR COMBINED MALE AND FEMALE FISCHER 344 RATS

C ACUTE ORAL LD50 RDX FISCHER 344 RATS COMBINED

FUNCTION OF DOSE -- NATURAL LOG

DOSE	SUBJECTS	RESPONSES	PERCENT RESPONSE	WEIGHT COEFF.	PROBIT	CONTRIBUTION TO CHI-SQUARE
150.0	20.	20.	100.00	.0540	7.46	.14
125.0	20.	14.	70.00	.5618	5.58	.04
100.0	20.	1.	5.00	.2032	3.28	.02

AFTER 3 ITERATIONS. PROBIT = 10.32 * LN(DOSE) + -44.24

DEGREES OF FREEDOM =	1
CHI-SQUARED =	.20
HETEROGENITY FACTOR =	1.00
T FACTOR =	1.96
PROBABILITY OF POOR FIT =	.318
FINNEY@S G FACTOR =	.174

		STANDARD ERROR	95 PERCENT CONFIDENCE LIMITS		
SLOPE	10.32	2.195	6.016	TO	14.62.
LD10	104.3	3.944	92.96	TO	110.5.
LD50	118.1	2.848	111.8	TO	124.1.
LD90	133.7	4.491	126.9	TO	147.7.

TABLE 4

PROBIT ANALYSIS OF MORTALITY DATA FOR FISCHER 344 MALE RATS

C ACUTE ORAL LD50 RDX IN FISCHER 344 RATS MALE

FUNCTION OF DOSE -- NATURAL LOG

DOSE	SUBJECTS	RESPONSES	PERCENT RESPONSE	WEIGHT COEFF.	PROBIT	CONTRIBUTION TO CHI-SQUARE
150.0	10.	10.	100.00	.0008	8.98	0.00
125.0	10.	8.	80.00	.4901	5.84	.00
100.0	10.	0.	0.00	.0151	1.99	0.00

AFTER 3 ITERATIONS. PROBIT = 17.25 * LN(DOSE) + -77.44

DEGREES OF FREEDOM = 1
CHI-SQUARED = .00
HETEROGENITY FACTOR = 1.00
T FACTOR = 1.96
PROBABILITY OF POOR FIT = .002
FINNEY@S G FACTOR = 1.704

WARNING: G GREATER THAN ONE. LIMITS ARE INVALIDLY BASED ON THE VARIANCE FORMULA, NOT FIELLER@S.

		STANDARD ERROR	95 PERCENT CONFIDENCE LIMITS		
SLOPE	17.25	11.49	-5.265	TO	39.76
LD10	110.5	9.056	94.15	TO	129.8
LD50	119.0	4.554	110.4	TO	128.3
LD90	128.2	4.276	120.1	TO	136.9

TABLE 5

PROBIT ANALYSIS OF MORTALITY DATA FOR
FISCHER 344 FEMALE RATS

C ACUTE ORAL LD50 RDX FISCHER 344 RATS FEMALE

FUNCTION OF DOSE -- NATURAL LOG

DOSE	SUBJECTS	RESPONSES	PERCENT RESPONSE	WEIGHT COEFF.	PROBIT	CONTRIBUTION TO CHI-SQUARE
150.0	10.	10.	100.00	.1410	6.96	.26
125.0	10.	6.	60.00	.5947	5.43	.20
100.0	10.	1.	10.00	.2906	3.57	.08

AFTER 5 ITERATIONS, PROBIT = 8.362 * LN(DOSE) + -34.94

DEGREES OF FREEDOM = 1
CHI-SQUARED = .54
HETEROGENITY FACTOR = 1.00
T FACTOR = 1.96
PROBABILITY OF POOR FIT = .497
FINNEY@S G FACTOR = .311

		STANDARD ERROR	95 PERCENT CONFIDENCE LIMITS		
SLOPE	8.362	2.379	3.698	TO	13.03.
LD10	101.9	6.149	80.42	TO	111.0.
LD50	118.7	4.456	108.0	TO	128.9.
LD90	138.3	7.544	127.7	TO	170.0.

TABLE 6

PROBIT ANALYSIS OF MORTALITY DATA FOR COMBINED MALE AND FEMALE B6C3F1 MICE

ACUTE ORAL LD50 OF RDX IN B6 MICE

FUNCTION OF DOSE -- NATURAL LOG

DOSE	SUBJECTS	RESPONSES	PERCENT RESPONSE	WEIGHT COFFF.	PROBIT	CONTRIBUTI[ON] TO CHI-SQUA[RE]
60.00	10.	3.	30.00	.5395	4.33	.13
100.0	10.	6.	60.00	.5797	5.51	.41
140.0	10.	9.	90.00	.3421	6.28	.00
180.0	10.	10.	100.00	.1643	6.86	.32

AFTER 4 ITERATIONS, PROBIT = 2.307 * LN(DOSE) + -5.117

DEGREES OF FREEDOM = 2
CHI-SQUARED = .86
HETEROGENITY FACTOR = 1.00
T FACTOR = 1.96
PROBABILITY OF POOR FIT = .345
FINNEY@S G FACTOR = .309

		STANDARD ERROR	95 PERCENT CONFIDENCE LIMITS		
SLOPE	2.307	.6541	1.025	TO	3.589
LD10	46.0'	10.91	17.45	TO	63.13
LD50	80.27	9.579	55.30	TO	99.17
LD90	139.8	21.20	111.5	TO	244.9

TABLE 7

PROBIT ANALYSIS OF MORTALITY DATA FOR
B6C3F1 MALE MICE

ACUTE ORAL LD50 RDX IN MICE MALE

FUNCTION OF DOSE -- NATURAL LOG

DOSE	SUBJECTS	RESPONSES	PERCENT RESPONSE	WEIGHT COEFF.	PROBIT	CONTRIBUTION TO CHI-SQUARE
140.0	5.	5.	100.00	.0058	8.24	0.00
100.0	5.	3.	60.00	.6218	5.25	.00
60.00	5.	0.	0.00	0.0000	.72	0.00

AFTER 4 ITERATIONS, PROBIT = 8.872 * LN(DOSE) + -35.60

DEGREES OF FREEDOM = 1
CHI-SQUARED = .00
HETEROGENITY FACTOR = 1.00
T FACTOR = 1.96
PROBABILITY OF POOR FIT = .002
FINNEY@S G FACTOR = 15.004

WARNING: G GREATER THAN ONE. LIMITS ARE INVALIDLY BASED ON THE VARIANCE FORMULA, NOT FIELLER@S.

		STANDARD ERROR	95 PERCENT CONFIDENCE LIMITS		
SLOPE	8.872	17.53	-25.49	TO	43.24
LD10	84.13	30.36	42.08	TO	168.2
LD50	97.19	8.685	81.59	TO	115.8
LD90	112.3	26.22	71.32	TO	176.7

TABLE 8

PROBIT ANALYSIS OF MORTALITY DATA FOR
B6C3F1 FEMALE MICE

ACUTE ORAL LD50 RDX IN MICE FEMALE

FUNCTION OF DOSE -- NATURAL LOG

DOSE	SUBJECTS	RESPONSES	PERCENT RESPONSE	WEIGHT COEFF.	PROBIT	CONTRIBUTION TO CHI-SQUARE
180.0	5.	5.	100.00	.3225	6.34	.50
140.0	5.	4.	80.00	.4257	6.04	.10
100.0	5.	3.	60.00	.5490	5.64	.49
60.00	5.	3.	60.00	.6365	5.02	.16

AFTER 5 ITERATIONS, PROBIT = 1.198 * LN(DOSE) + .1163

DEGREES OF FREEDOM = 2
CHI-SQUARED = 1.25
HETEROGENITY FACTOR = 1.00
T FACTOR = 1.96
PROBABILITY OF POOR FIT = .457
FINNEY@S G FACTOR = 1.643

WARNING: G GREATER THAN ONE. LIMITS ARE INVALIDLY BASED ON THE VARIANCE FORMULA, NOT FIELLER@S.

		STANDARD ERROR	95 PERCENT CONFIDENCE LIMITS		
SLOPE	1.198	.7836	-.3375	TO	2.734
LD10	20.23	26.39	2.429	TO	168.5
LD50	58.86	26.77	24.83	TO	139.5
LD90	171.3	77.88	72.24	TO	406.0

TABLE 9

BODY WEIGHTS (g) OF MALE RATS FED RDX

Test Week	Control[b/]	Dose Group (mg/kg/day) 10	14	20	28	40
Initial	86.1 ± 1.6	86.2 ± 1.8	88.0 ± 1.8	86.4 ± 1.8	87.1 ± 2.0	86.1 ± 2.1
1	124.0 ± 2.2	125.0 ± 2.7	124.4 ± 1.7	121.9 ± 3.4	121.2 ± 2.2	116.1 ± 1.5[a/]
2	154.7 ± 2.6	162.9 ± 4.8	155.3 ± 2.0	149.0 ± 5.0	145.4 ± 2.8	138.0 ± 2.1
3	183.9 ± 4.9	186.3 ± 5.1	183.8 ± 5.0	178.9 ± 6.9	172.7 ± 3.5	163.4 ± 2.3[a/]
4	205.5 ± 3.1	213.0 ± 3.4	208.2 ± 3.1	199.3 ± 8.0	195.3 ± 3.1	184.6 ± 3.6[a/]
5	232.1 ± 3.0	233.9 ± 3.5	231.1 ± 2.7	222.9 ± 8.7	216.1 ± 2.9	200.0 ± 5.8[a/]
6	244.4 ± 3.1	244.1 ± 4.5	244.1 ± 2.6	237.2 ± 8.9	227.8 ± 4.4	218.4 ± 4.0[a/]
7	254.1 ± 3.8	257.7 ± 4.2	257.1 ± 2.3	252.2 ± 9.3	242.6 ± 3.4	231.4 ± 3.5[a/]
8	268.1 ± 3.2	268.0 ± 4.5	269.3 ± 2.3	264.4 ± 9.2	259.5 ± 2.8	243.8 ± 3.9[a/]
9	278.7 ± 3.5	280.6 ± 3.9	279.6 ± 2.3	275.6 ± 9.2	268.3 ± 3.3	252.9 ± 3.8[a/]
10	282.5 ± 3.2	281.7 ± 3.9	283.5 ± 2.7	280.0 ± 10.2	269.8 ± 3.9	254.4 ± 4.1[a/]
11	295.6 ± 3.8	295.5 ± 4.7	296.5 ± 3.1	289.4 ± 9.7	280.9 ± 3.8	266.8 ± 3.7[a/]
12	298.3 ± 3.2	299.9 ± 4.8	298.3 ± 3.0	292.5 ± 8.5	284.2 ± 2.8	271.7 ± 3.9[a/]
13	306.2 ± 3.7	308.4 ± 5.2	308.3 ± 2.7	303.3 ± 10.0	295.7 ± 3.0	280.6 ± 3.3[a/]

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.
b/ Entries are mean ± standard error of ten animals.

TABLE 10

BODY WEIGHT (g) OF FEMALE RATS FED RDX

Test Week	Control[b]	Dose Group (mg/kg/day) 10	14	20	28	40
Initial	61.4 ± 1.3	60.5 ± 1.5	61.3 ± 1.2	61.5 ± 1.3	61.0 ± 1.3	61.8 ± 1.3
1	89.8 ± 1.6	90.3 ± 2.1	91.7 ± 1.5	91.2 ± 1.2	86.2 ± 1.5	89.3 ± 1.5
2	104.5 ± 2.1	103.8 ± 2.6	103.4 ± 1.8	100.1 ± 1.6	92.9 ± 1.5[a]	99.4 ± 2.0
3	119.3 ± 2.5	117.9 ± 3.7	115.7 ± 2.7	119.4 ± 1.5	113.1 ± 2.6	116.7 ± 2.3
4	128.0 ± 3.1	128.0 ± 2.9	127.4 ± 2.4	128.0 ± 1.5	122.9 ± 2.6	124.9 ± 2.6
5	139.8 ± 2.6 (9)	139.8 ± 2.8	139.1 ± 2.0	138.8 ± 1.7	133.3 ± 3.6	135.1 ± 2.8
6	147.6 ± 2.4 (9)	147.7 ± 3.0	146.6 ± 1.9	146.6 ± 2.0	139.5 ± 3.4	142.4 ± 2.9
7	153.4 ± 2.3 (9)	150.3 ± 2.5	151.8 ± 1.7	152.8 ± 1.5	146.5 ± 3.2	149.8 ± 2.8
8	158.6 ± 2.1 (9)	158.5 ± 3.4	157.5 ± 1.8	158.2 ± 2.2	153.1 ± 3.2	155.7 ± 3.2
9	161.8 ± 2.9 (9)	162.6 ± 3.3	160.7 ± 2.1	161.3 ± 1.7	155.2 ± 3.0	157.3 ± 2.8
10	163.2 ± 2.7 (9)	161.8 ± 2.9	162.1 ± 1.9	161.0 ± 1.6	155.1 ± 2.8	156.9 ± 3.0
11	172.8 ± 2.7 (9)	170.1 ± 3.2	168.9 ± 2.2	169.6 ± 2.1	164.1 ± 2.8	164.5 ± 3.3
12	174.1 ± 2.9 (9)	171.9 ± 3.0	170.8 ± 2.0	171.1 ± 2.2	165.1 ± 3.0	166.5 ± 3.1
13	178.7 ± 2.6 (9)	176.3 ± 3.4	175.6 ± 1.7	175.5 ± 2.2	171.1 ± 3.1	168.8 ± 3.7

a/ Statistically different from control group, $p < 0.05$, Dunnett's multiple comparison.
b/ Entries are mean ± standard error of ten animals except as noted in parenthesis.

TABLE 11

FEED CONSUMPTION (g/day) IN MALE RATS FED RDX

Test Week	Control[b/]	Dose Group (mg/kg/day)				
		10	14	20	28	40
1	14.0 ± 0.1	14.4 ± 0.3	13.2 ± 0.9	14.1 ± 0.5	12.4 ± 0.4	11.6 ± 0.5[a/]
2	16.5 ± 0.2	16.8 ± 0.6	16.4 ± 0.3	16.1 ± 0.8	15.3 ± 0.3	14.8 ± 0.3
3	17.9 ± 0.1	18.6 ± 0.6	15.6 ± 1.6	17.2 ± 0.9	16.5 ± 0.2	16.1 ± 0.5
4	18.8 ± 0.2	19.2 ± 0.5	18.7 ± 0.3	17.7 ± 0.9	17.3 ± 0.3	16.6 ± 0.7
5	18.9 ± 0.2	19.2 ± 0.3	18.7 ± 0.3	18.4 ± 0.8	17.8 ± 0.2	15.9 ± 1.6
6	18.5 ± 0.2	18.8 ± 0.3	18.7 ± 0.3	18.6 ± 0.9	16.7 ± 1.0	17.2 ± 0.7
7	18.6 ± 0.1	18.1 ± 0.3	18.0 ± 0.4	18.2 ± 0.7	17.5 ± 0.2	16.6 ± 0.4[a/]
8	18.7 ± 0.3	18.7 ± 0.3	19.1 ± 0.3	19.3 ± 0.7	18.2 ± 0.3	17.4 ± 0.5
9	18.2 ± 0.3	18.4 ± 0.3	18.1 ± 0.1	17.7 ± 0.5	17.5 ± 0.3	16.7 ± 0.5
10	18.3 ± 0.2	18.4 ± 0.3	17.9 ± 0.2	17.6 ± 0.7	16.9 ± 0.3	15.9 ± 0.4[a/]
11	18.7 ± 0.3	19.1 ± 0.4	18.7 ± 0.3	18.4 ± 0.6	17.4 ± 0.3	16.7 ± 0.4[a/]
12	18.6 ± 0.2	18.8 ± 0.4	18.3 ± 0.5	18.2 ± 0.5	17.5 ± 0.3	16.8 ± 0.5
13	18.4 ± 0.2	18.4 ± 0.3	18.3 ± 0.4	18.0 ± 0.5	17.4 ± 0.3	16.5 ± 0.3[a/]

a/ Statistically significant from control group, $p < 0.05$, Dunnett's multiple comparison.
b/ Entries are mean ± standard error of five cages.

TABLE 12

FEED CONSUMPTION (g/day) IN FEMALE RATS FED RDX

Test Week	Control a/	Dose Group (mg/kg/day) 10	14	20	28	40
1	10.4 ± 0.5	11.2 ± 0.3	11.0 ± 0.4	10.5 ± 0.7	9.7 ± 0.7	8.5 ± 1.3
2	12.2 ± 0.3	12.4 ± 0.4	12.3 ± 0.2	12.2 ± 0.3	11.4 ± 0.3	11.3 ± 0.2
3	12.5 ± 0.2	12.3 ± 0.4	12.2 ± 0.3	12.8 ± 0.5	12.1 ± 0.4	11.7 ± 0.2
4	12.0 ± 0.3	13.0 ± 0.4	12.3 ± 0.4	12.8 ± 0.5	12.4 ± 0.4	11.9 ± 0.3
5	11.2 ± 0.9 (9)	12.6 ± 0.3	12.5 ± 0.4	12.6 ± 0.5	12.2 ± 0.4	11.8 ± 0.1
6	12.5 ± 0.2 (9)	13.1 ± 0.3	13.3 ± 0.3	13.5 ± 0.6	12.4 ± 0.4	12.0 ± 0.3
7	12.7 ± 0.3 (9)	12.5 ± 0.3	12.2 ± 0.4	12.6 ± 0.5	11.9 ± 0.4	12.0 ± 0.2
8	13.0 ± 0.3 (9)	13.7 ± 0.4	13.2 ± 0.3	13.9 ± 0.6	12.8 ± 0.4	12.5 ± 0.3
9	12.8 ± 0.3 (9)	12.4 ± 0.3	12.2 ± 0.4	12.2 ± 0.4	11.9 ± 0.3	11.6 ± 0.2
10	12.7 ± 0.4 (9)	12.8 ± 0.3	12.7 ± 0.4	12.8 ± 0.5	12.1 ± 0.3	11.6 ± 0.3
11	13.2 ± 0.3 (9)	13.1 ± 0.2	12.7 ± 0.4	13.2 ± 0.6	12.2 ± 0.5	11.8 ± 0.3
12	13.7 ± 0.4 (9)	13.1 ± 0.3	12.8 ± 0.4	13.2 ± 0.7	12.5 ± 0.4	12.1 ± 0.3
13	13.0 ± 0.4 (9)	12.9 ± 0.3	12.7 ± 0.3	13.4 ± 0.6	12.6 ± 0.5	12.3 ± 0.3

a/ Entries are mean ± standard error of five cages except as noted in parenthesis.

TABLE 13

CALCULATED RDX INTAKE (mg/kg/day) IN MALE MICE

Test Week	Control[a]	Dose Group (mg/kg/day) 10	14	20	28	40
1	0	13.7 ± 0.3	17.4 ± 0.9	27.1 ± 0.2	33.3 ± 1.3	45.9 ± 1.4
2	0	8.1 ± 0.2	12.5 ± 0.2	16.7 ± 0.3	25.7 ± 0.2	38.8 ± 0.6
3	0	8.5 ± 0.3	10.3 ± 1.0	16.7 ± 0.2	23.6 ± 0.2	35.3 ± 0.7
4	0	8.8 ± 0.2	14.2 ± 0.3	17.4 ± 0.0	24.9 ± 0.4	35.0 ± 0.8
5	0	8.4 ± 0.1	12.0 ± 0.2	17.6 ± 0.1	24.0 ± 0.3	33.5 ± 2.6
6	0	8.7 ± 0.2	12.1 ± 0.2	17.5 ± 0.1	22.8 ± 1.0	37.7 ± 1.8
7	0	9.1 ± 0.2	12.7 ± 0.2	18.3 ± 0.1	27.6 ± 0.5	34.7 ± 0.3
8	0	9.8 ± 0.1	14.1 ± 0.2	19.9 ± 0.3	27.0 ± 0.3	39.2 ± 0.4
9	0	9.4 ± 0.2	12.6 ± 0.1	17.5 ± 0.2	25.3 ± 0.2	36.5 ± 0.4
10	0	9.7 ± 0.2	13.4 ± 0.1	19.2 ± 0.3	26.4 ± 0.3	37.1 ± 0.6
11	0	10.1 ± 0.1	14.1 ± 0.1	20.4 ± 0.3	28.2 ± 0.3	40.9 ± 0.4
12	0	9.5 ± 0.1	13.3 ± 0.2	19.3 ± 0.3	27.4 ± 0.6	38.6 ± 0.6
13	0	9.1 ± 0.1	13.0 ± 0.1	18.6 ± 0.3	26.6 ± 0.5	36.9 ± 0.4
1-13[b]	0	9.5 ± 0.4	13.2 ± 0.5	19.0 ± 0.8	26.4 ± 0.7	37.7 ± 0.9

a/ Entries are mean ± standard error of 10 animals, based on nominal concentrations.
b/ Mean ± standard error of weekly means.

TABLE 14

CALCULATED RDX INTAKE (mg/kg/day) IN FEMALE RATS

Test Week	Control[a/]	Dose Group (mg/kg/day) 10	14	20	28	40
1	0	14.9 ± 0.4	20.1 ± 0.6	27.6 ± 1.7	36.8 ± 2.4	44.9 ± 6.6
2	0	8.1 ± 0.2	11.5 ± 0.1	17.4 ± 0.3	25.9 ± 0.4	40.7 ± 0.5
3	0	8.8 ± 0.1	12.6 ± 0.3	18.6 ± 0.6	26.7 ± 0.5	35.9 ± 0.5
4	0	9.4 ± 0.4	12.5 ± 0.3	17.3 ± 0.5	24.4 ± 0.6	35.6 ± 0.4
5	0	9.2 ± 0.3 (9)	13.2 ± 0.3	19.0 ± 0.5	26.4 ± 0.7	37.0 ± 0.8
6	0	10.0 ± 0.2 (9)	14.4 ± 0.2	20.6 ± 0.6	27.6 ± 0.6	39.5 ± 0.6
7	0	9.2 ± 0.2 (9)	12.2 ± 0.3	17.6 ± 0.5	25.6 ± 0.4	38.4 ± 0.3
8	0	12.1 ± 0.3 (9)	16.5 ± 0.2	23.8 ± 0.8	31.9 ± 0.6	43.8 ± 0.2
9	0	10.8 ± 0.2 (9)	14.6 ± 0.4	20.2 ± 0.6	29.5 ± 0.7	40.4 ± 0.4
10	0	11.6 ± 0.2 (9)	16.6 ± 0.4	24.0 ± 0.7	32.9 ± 0.5	43.6 ± 0.7
11	0	12.1 ± 0.2 (9)	16.8 ± 0.4	25.1 ± 1.0	34.2 ± 1.0	46.9 ± 0.5
12	0	11.6 ± 0.3 (9)	16.3 ± 0.3	23.8 ± 0.9	33.4 ± 0.7	45.1 ± 0.3
13	0	11.2 ± 0.2 (9)	15.8 ± 0.2	23.9 ± 0.6	32.9 ± 1.0	45.5 ± 0.6
1-13[b/]	0	10.7 ± 0.5	14.9 ± 0.7	21.5 ± 0.9	29.9 ± 1.1	41.3 ± 1.1

a/ Entries are mean ± standard error of 10 animals except as noted in parenthesis, based on nominal concentrations.

b/ Mean ± standard error of weekly means.

TABLE 15

LABORATORY DATA OF MALE RATS AFTER FEEDING OF RDX FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C, 5)	28 (T, 5)	40 (T, 5)
ERYTHROCYTES ($X10^6/MM^3$)	7.32 ± .14	7.13 ± .12	6.93 ± .21
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.02 ± .14	.99 ± .16	1.25 ± .22
HEMATOCRIT, VOL. %	52.4 ± 1.2	50.0 ± .5	48.6 ± 1.1 a/
HEMOGLOBIN, GM. %	17.6 ± .3	16.9 ± .2	16.4 ± .4 a/
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	71.6 ± 1.0	70.1 ± .7	70.2 ± .6
MCHB, MICRO MICROGMS.	24.1 ± .3	23.6 ± .3	23.7 ± .2
MCHBC, GM %	33.6 ± .2	33.7 ± .2	33.7 ± .2
PLATELETS ($X10^5/MM^3$)	7.4 ± 1.0	8.1 ± .7	8.1 ± .7
LEUKOCYTES ($X10^3/MM^3$)	13.7 ± .5	13.0 ± .5	13.8 ± .5
NEUTROPHILS, %	18.2 ± 2.1	17.4 ± 1.3	14.0 ± 1.3
LYMPHOCYTES, %	80.8 ± 2.6	81.6 ± 1.8	84.6 ± 1.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .4	.8 ± .6	.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.2 ± .2	.2 ± .2	.6 ± .4
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure.

TABLE 16

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF RDX FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C, 5)	28 (T, 5)	40 (T, 5)
ERYTHROCYTES ($X10^6/MM^3$)	7.49 ± .17	7.80 ± .17	7.33 ± .16
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.43 ± .02	1.13 ± .18 a/	.91 ± .10
HEMATOCRIT, VOL. %	55.0 ± .8	54.8 ± 1.1	53.6 ± .4
HEMOGLOBIN, GM. %	18.4 ± .3	18.3 ± .3	18.0 ± .2
METHEMOGLOBIN, %	.3 ± .3	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	73.5 ± .6	70.3 ± .5 a/	73.2 ± 1.1
MCHB, MICRO MICROGMS	24.5 ± .2	23.5 ± .2 a/	24.5 ± .3
MCHBC, GM %	33.4 ± .1	33.4 ± .2	33.5 ± .1
PLATELETS ($X10^5/MM^3$)	6.9 ± 1.0	9.2 ± .7 (4)	7.0 ± .3
LEUKOCYTES ($X10^3/MM^3$)	12.8 ± .5	14.4 ± .9	15.3 ± .3 a/
NEUTROPHILS, %	9.4 ± 2.3	10.0 ± 1.3	12.2 ± 1.9
LYMPHOCYTES, %	89.4 ± 2.0	88.4 ± 1.6	87.4 ± 2.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .5	1.2 ± .4	.4 ± .2
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.2 ± .2	.4 ± .2	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR OF 5 ANIMALS EXCEPT AS NOTED IN PARENTHESIS

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure.

TABLE 17

LABORATORY DATA OF MALE RATS AFTER FEEDING OF RDX FOR 2 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
ERYTHROCYTES $(X10^6/MM^3)$	7.58 ± .14	7.40 ± .08	7.21 ± .11
HEINZ BODIES.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES. %	.85 ± .20	.59 ± .22 (4)	.98 ± .11
HEMATOCRIT. VOL. %	49.8 ± .5	48.8 ± .4	47.8 ± .6 a/
HEMOGLOBIN. GM. %	16.8 ± .1	16.5 ± .1	16.1 ± .2 a/
METHEMOGLOBIN.	0.0 ± 0.0	.3 ± .3	.3 ± .3
MCV. CUBIC MICRONS	65.7 ± .6	65.9 ± .4	66.3 ± .3
MCHB. MICRO MICROGMS.	22.2 ± .2	22.3 ± .2	22.3 ± .1
MCHBC. GM %	33.8 ± .1	33.8 ± .1	33.6 ± .1
PLATELETS $(X10^5/MM^3)$	5.0 ± .3	4.7 ± .2	5.4 ± .3
LEUKOCYTES $(X10^3/MM^3)$	12.4 ± 2.3	12.6 ± .5	13.5 ± .8
NEUTROPHILS. %	14.8 ± 1.7	15.0 ± 3.4	14.4 ± 2.2
LYMPHOCYTES. %	83.2 ± 1.9	83.6 ± 3.1	84.0 ± 2.6
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	1.2 ± .5	.4 ± .2	.8 ± .6
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	.8 ± .2	1.0 ± .3	.8 ± .2
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR OF 5 ANIMALS EXCEPT AS NOTED IN PARENTHESIS

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure.

TABLE 18

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF RDX FOR 2 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C, 5)	28 (T, 5)	40 (T, 5)
ERYTHROCYTES ($X10^6/MM^3$)	7.49 ± .21 (4)	7.47 ± .09	7.08 ± .13
HEINZ BODIES,	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.71 ± .19 (4)	1.36 ± .13	.59 ± .12 a/
HEMATOCRIT, VOL. %	50.5 ± .6 (4)	50.4 ± .4	49.4 ± .5
HEMOGLOBIN, GM, %	17.2 ± .3 (4)	17.0 ± .2	16.7 ± .2
METHEMOGLOBIN,	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	67.5 ± 1.1 (4)	67.4 ± .3	69.9 ± .8
MCHB, MICRO MICROGMS,	22.9 ± .3 (4)	22.8 ± .1	23.6 ± .2
MCHBC, GM %	34.0 ± .3 (4)	33.8 ± .1	33.8 ± .1
PLATELETS ($X10^5/MM^3$)	4.5 ± .2 (4)	4.6 ± .3	5.3 ± .3
LEUKOCYTES ($X10^3/MM^3$)	13.1 ± .2 (4)	12.7 ± .5	14.2 ± .5
NEUTROPHILS, %	14.8 ± 7.4	15.6 ± 2.8	10.8 ± 2.2
LYMPHOCYTES, %	63.6 ± 17.2	83.4 ± 2.9	88.2 ± 2.5
BANDS, %	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.6 ± .2	.8 ± .5	.6 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .4	.2 ± .2	.4 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR OF 5 ANIMALS EXCEPT AS NOTED IN PARENTHESIS

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure.

TABLE 19

LABORATORY DATA OF MALE RATS AFTER FEEDING OF RDX FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
ERYTHROCYTES ($X10^6/MM^3$)	7.32 ± .08	7.52 ± .13	7.25 ± .05
HEINZ BODIES.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES. %	.72 ± .08	.91 ± .10	1.27 ± .18 a/
HEMATOCRIT. VOL. %	49.2 ± .6	50.8 ± .7	49.0 ± .3
HEMOGLOBIN. GM. %	16.7 ± .2	17.1 ± .2	16.6 ± .1
METHEMOGLOBIN.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV. CUBIC MICRONS	67.2 ± .6	67.6 ± .8	67.6 ± .2
MCHB. MICRO MICROGMS.	22.8 ± .1	22.7 ± .3	22.9 ± .2
MCHBC. GM %	34.0 ± .2	33.6 ± .1	33.9 ± .2
PLATELETS ($X10^5/MM^3$)	4.5 ± .2	5.0 ± .2	5.2 ± .2 a/
LEUKOCYTES ($X10^3/MM^3$)	13.9 ± .4	12.2 ± .3	14.9 ± 1.3
NEUTROPHILS. %	24.0 ± 4.4	14.4 ± 1.3	16.4 ± 3.5
LYMPHOCYTES. %	73.8 ± 4.4	84.2 ± 1.5	81.6 ± 3.4
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	1.6 ± .6	.8 ± .4	1.4 ± .4
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	.6 ± .4	.6 ± .2	.6 ± .4
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Statistically different from control group, $p < 0.05$, Dunnett's multiple comparison procedure

TABLE 20

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF RDX FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
ERYTHROCYTES ($X10^6/MM^3$)	7.34 ± .25 (4)	7.26 ± .06	6.84 ± .24
HEINZ BODIES,	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.21 ± .17 (4)	1.19 ± .09	1.41 ± .09
HEMATOCRIT, VOL. %	50.5 ± 1.7 (4)	50.4 ± .2	49.6 ± .6
HEMOGLOBIN, GM. %	17.1 ± .6 (4)	17.0 ± .1	16.9 ± .2
METHEMOGLOBIN,	.2 ± .2	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	68.8 ± 1.5 (4)	69.5 ± .8	72.8 ± 2.7
MCHB, MICRO MICROGMS.	23.4 ± .5 (4)	23.4 ± .3	24.8 ± .9
MCHBC, GM %	34.0 ± .1 (4)	33.7 ± .1	34.0 ± .1
PLATELETS ($X10^5/MM^3$)	5.6 ± 1.3 (4)	4.3 ± .4	4.9 ± .2
LEUKOCYTES ($X10^3/MM^3$)	11.5 ± .9 (4)	13.4 ± 1.1	14.9 ± 1.1
NEUTROPHILS, %	13.0 ± 5.0	14.6 ± 1.5	14.6 ± 3.7
LYMPHOCYTES, %	65.6 ± 16.9	84.2 ± 1.7	84.2 ± 3.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .4	.4 ± .2	1.0 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.4 ± .2	.8 ± .5	.2 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR OF 5 ANIMALS EXCEPT AS NOTED IN PARENTHESIS

TABLE 21

LABORATORY DATA OF MALE RATS AFTER FEEDING OF RDX FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
GLUCOSE (FASTING), MG %	111.0 ± 4.2	106.0 ± 2.1	99.4 ± 3.1 a/
SGOT, IU/L	72.2 ± 3.9	68.6 ± 3.7	67.4 ± 1.7
SGPT, IU/L	28.6 ± .6	25.6 ± 1.0 a/	24.8 ± .9 a/
ALK. PHOS., IU/L	84 ± 4	81 ± 4	81 ± 3
BUN, MG %	17.2 ± 1.5	17.6 ± .4	16.2 ± .7
SODIUM, MEQ/L	147 ± 1	147 ± 1	147 ± 1
POTASSIUM, MEQ/L	5.0 ± .0	4.9 ± .1	4.8 ± .0 a/
CALCIUM, MEQ/L	5.0 ± .1	4.9 ± .1	5.0 ± .1

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure

TABLE 22

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF RDX FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF RATS

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
GLUCOSE (FASTING), MG %	109.4 ± 2.8	109.4 ± 6.0	107.8 ± 3.8
SGOT, IU/L	60.0 ± 2.4	56.0 ± 1.3	51.6 ± 2.2 a/
SGPT, IU/L	27.0 ± 1.3	26.2 ± 2.2	25.6 ± 1.7
ALK. PHOS., IU/L	91 ± 17	84 ± 5	77 ± 5
BUN, MG %	17.0 ± .8	19.2 ± .6	17.0 ± .6
SODIUM, MEQ/L	147 ± 1	150 ± 1 a/	148 ± 1
POTASSIUM, MEQ/L	4.5 ± .1	4.4 ± .1	4.5 ± .1
CALCIUM, MEQ/L	4.7 ± .1	4.8 ± .0	4.8 ± .1

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure

TABLE 23

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED RDX FOR 90 DAYS[a/]

Sex	Dose (mg/kg/day)	Terminal Body Weight (g)	Absolute Organ Weight (g): Brain	Heart	Liver	Kidney	Spleen	Gonad
Male	0	306.2 ± 3.7	1.88 ± 0.02	0.95 ± 0.02	9.7 ± 0.6	2.26 ± 0.05	0.57 ± 0.01	3.01 ± 0.04
	28	295.7 ± 3.0	1.93 ± 0.02	0.95 ± 0.03	9.5 ± 0.5	2.21 ± 0.05	0.58 ± 0.02	2.96 ± 0.04
	40	280.6 ± 3.3[b/]	1.88 ± 0.01	0.87 ± 0.02[b/]	9.2 ± 0.4	2.18 ± 0.04	0.55 ± 0.01	3.01 ± 0.02
Female	0	173.1 ± 6.0	1.77 ± 0.02 (9)	0.65 ± 0.03 (9)	5.2 ± 0.3 (9)	1.41 ± 0.02 (9)	0.42 ± 0.02 (9)	0.12 ± 0.01 (9)
	28	171.1 ± 3.1	1.77 ± 0.01	0.61 ± 0.01	5.5 ± 0.3	1.43 ± 0.04	0.38 ± 0.02 (9)	0.16 ± 0.01
	40	168.8 ± 3.7	1.77 ± 0.02	0.58 ± 0.01[b/]	5.4 ± 0.2	1.41 ± 0.04	0.37 ± 0.01[b/]	0.15 ± 0.02

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/100 g body weight): Brain	Heart	Liver	Kidney	Spleen	Gonad
Male	0	0.61 ± 0.01	0.31 ± 0.00	3.17 ± 0.19	0.74 ± 0.01	0.19 ± 0.00	0.98 ± 0.01
	28	0.65 ± 0.01[b/]	0.32 ± 0.01	3.22 ± 0.16	0.75 ± 0.02	0.20 ± 0.01	1.00 ± 0.01
	40	0.67 ± 0.01[b/]	0.31 ± 0.01	3.28 ± 0.17	0.78 ± 0.01	0.20 ± 0.00	1.07 ± 0.02[b/]
Female	0	0.99 ± 0.01 (9)	0.37 ± 0.02 (9)	2.88 ± 0.18 (9)	0.79 ± 0.01 (9)	0.24 ± 0.01 (9)	0.07 ± 0.01 (9)
	28	1.04 ± 0.02	0.36 ± 0.01	3.18 ± 0.17	0.84 ± 0.02	0.22 ± 0.01	0.09 ± 0.01
	40	1.05 ± 0.03	0.34 ± 0.01	3.21 ± 0.14	0.84 ± 0.02	0.22 ± 0.01	0.09 ± 0.01

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/g brain weight): Heart	Liver	Kidney	Spleen	Gonad
Male	0	0.51 ± 0.01	5.2 ± 0.3	1.20 ± 0.03	0.31 ± 0.01	1.61 ± 0.03
	28	0.49 ± 0.01	4.9 ± 0.3	1.15 ± 0.02	0.30 ± 0.01	1.54 ± 0.02
	40	0.46 ± 0.01[b/]	4.9 ± 0.3	1.16 ± 0.02	0[illegible] ± 0.01	1.60 ± 0.02
Female	0	0.37 ± 0.01 (9)	2.9 ± 0.2 (9)	0.80 ± 0.02 (9)	0.24 ± 0.01 (9)	0.07 ± 0.01 (9)
	28	0.35 ± 0.01	3.1 ± 0.2	0.81 ± 0.02	0.21 ± 0.01 (9)	0.09 ± 0.01
	40	0.33 ± 0.01[b/]	3.1 ± 0.2	0.80 ± 0.03	0.21 ± 0.01[b/]	0.09 ± 0.01

a/ Mean ± standard error of ten rats except as noted in parenthesis.
b/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.

TABLE 24

SUMMARY OF LESIONS IN MALE RATS FED RDX FOR 90 DAYS

Dose (mg/kg/day):	0.0										40.0									
Lesions[a] Rat No.:	180	181	182	183	184	185	186	187	188	189	230	231	232	233	234	235	236	237	238	239
Eye																				
Focal proliferation of corneal epithelium																			±	
Lung																				
Parabronchiolar mononuclear cell aggregation	1	1	1	1	1		1		1	1	1	1	1	1	1	1	1	1	1	1
Perivascular edema								1												
Heart																				
Foci of proliferating endomysial cells	±																		±	
Foci of myocardial degeneration				±		±	±							±						
Liver																				
Pedunculated liver nodule (anomaly)																X				
Bile duct hyperplasia	±	±	±	±	±		±				±	±								
Portal inflammation	±			±								±		±				±		
Cytoplasmal alteration																				
Centrilobular																	1		±	±
Periportal							±											±		
Diffuse						±		±	±	±										
Granulomas													1							±
Intestine																				
Parasitism (pinworm)	X		X		X				X	X	X	X		X	X		X	X	X	X
Stomach																				
Calcification of mucosa		1	1																	
Testis																				
Atrophy of seminiferous tubules			1																	
Kidney																				
Foci of mononuclear cells							±			±				±				±		±
Distended tubule (focal)															±					

a/ Severity of lesions: ± - minimum; 1 - mild; X - the lesion is present.

TABLE 25

SUMMARY OF LESIONS IN FEMALE RATS FED RDX FOR 90 DAYS

Lesions[a/] Dose (mg/kg/day)	0.0										40.0									
Rat No.:	240	241	242	243	244	245	246	247	248	249	290	291	292	293	294	295	296	297	298	299
Trachea																				
Tracheitis								1			1	1								
Lung																				
Parabronchiolar mononuclear cell aggregation	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Focal pulmonary edema												1								
Heart																				
Foci of proliferating endomysial cells				±	±					±	±				±					
Foci of myocardial degeneration						±	±						±	±	±			±	±	±
Liver																				
Bile duct hyperplasia	±		±	±	±							±								
Portal inflammation							±				±		±			±	±	±	±	±
Cytoplasmic alteration																				
Centrilobular									±							±				±
Intestine																				
Parasitism (pinworm)				X					X			X	X		X			X		
Stomach																				
Calcification																	1	1	1	
Kidney																				
Foci of mononuclear cells																	1			
Dilated tubules																±				
Microcalculi in pelvis																		±		

a/ Severity of lesions: ± - minimum; 1 - mild; X - the lesion is present.

TABLE 26

BODY WEIGHTS (g) OF MALE MICE FED RDX

Test Week	Control[b]	Dose Group (mg/kg/day) 10	14	20	28	40
Initial	23.9 ± 0.6	24.2 ± 0.7	23.7 ± 0.7	24.6 ± 0.7	24.3 ± 0.6	24.2 ± 0.7
1	25.6 ± 0.6	25.0 ± 0.6	26.4 ± 0.9	25.8 ± 0.5	25.8 ± 0.6	25.3 ± 0.6
2	25.2 ± 0.4	27.2 ± 0.2[a]	26.0 ± 0.6	26.6 ± 0.4	25.5 ± 0.5	26.3 ± 0.4
3	[illegible]	25.8 ± 0.5	27.2 ± 0.6	27.2 ± 0.5	27.6 ± 0.7	27.7 ± 0.6
4	[illegible] ± 0.5	25.3 ± 0.5	26.6 ± 0.6	26.5 ± 0.4	26.3 ± 0.4	26.7 ± 0.5
5	26.8 ± 0.7	26.5 ± 0.4	26.7 ± 0.5	27.3 ± 0.4	27.6 ± 0.3	27.6 ± 0.5
6	27.1 ± 0.6	26.8 ± 0.5	27.2 ± 0.6	27.8 ± 0.4	27.6 ± 0.3	[illegible] ± 0.5
7	27.8 ± 0.6	27.5 ± 0.5	28.0 ± 0.6	27.7 ± 0.4	28.0 ± 0.3	27.3 ± 0.5
8	27.5 ± 0.5	27.6 ± 0.5	27.5 ± 0.6	27.7 ± 0.4	27.9 ± 0.3	27.4 ± 0.4
9	27.6 ± 0.6	27.5 ± 0.4	28.0 ± 0.6	28.0 ± 0.4	26.9 ± 0.9	27.8 ± 0.5
10	27.9 ± 0.5	27.9 ± 0.5	27.8 ± 0.6	27.7 ± 0.5	27.6 ± 0.4 (9)	27.7 ± 0.5
11	27.7 ± 0.6	27.4 ± 0.6	27.2 ± 0.7	27.5 ± 0.5	27.3 ± 0.4 (9)	27.4 ± 0.6
12	28.2 ± 0.6	28.0 ± 0.5	28.3 ± 0.7	[illegible] ± 0.4	29.7 ± 0.4 (9)	27.9 ± 0.5
13	28.5 ± 0.6	28.5 ± 0.6	28.4 ± 0.7	28.5 ± 0.5	28.3 ± 0.5 (9)	28.6 ± 0.6

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.
b/ Entries are mean ± standard error of ten animals except as noted in parenthesis.

TABLE 27

BODY WEIGHTS (g) OF FEMALE MICE FED RDX

Test Week	Control[a]	Dose Group (mg/kg/day) 10	14	20	28	40
Initial	19.5 ± 0.3	19.5 ± 0.4	19.9 ± 0.4	19.6 ± 0.3	19.7 ± 0.4	19.8 ± 0.4
1	20.4 ± 0.4	20.5 ± 0.5	21.7 ± 0.5	20.3 ± 0.4	20.9 ± 0.3	20.6 ± 0.5
2	21.7 ± 0.5	22.6 ± 0.5	22.2 ± 0.4	21.2 ± 0.4	21.8 ± 0.3	21.1 ± 0.3
3	22.5 ± 0.4	23.3 ± 0.4	22.5 ± 0.3	22.4 ± 0.4	22.6 ± 0.3	22.9 ± 0.2
4	21.8 ± 0.4	22.5 ± 0.6	22.7 ± 0.4	22.2 ± 0.3	22.3 ± 0.2	23.0 ± 0.3
5	23.2 ± 0.3	24.4 ± 0.3	24.2 ± 0.4	23.3 ± 0.4	23.5 ± 0.3	23.2 ± 0.3
6	23.7 ± 0.5	23.9 ± 0.4	24.2 ± 0.4	24.5 ± 0.6	23.9 ± 0.2	23.5 ± 0.3
7	23.9 ± 0.5	24.8 ± 0.5	24.7 ± 0.3	24.3 ± 0.4	24.0 ± 0.3	24.0 ± 0.4
8	24.4 ± 0.5	25.1 ± 0.5	25.5 ± 0.5	24.4 ± 0.4	24.1 ± 0.3	23.9 ± 0.3
9	23.9 ± 0.5	25.7 ± 0.5	25.3 ± 0.5	24.4 ± 0.5	24.5 ± 0.3	24.8 ± 0.4
10	24.7 ± 0.4	25.3 ± 0.4	25.5 ± 0.5	24.9 ± 0.4	24.5 ± 0.3	24.7 ± 0.4
11	24.8 ± 0.5	25.5 ± 0.5	25.4 ± 0.6	25.0 ± 0.5	24.7 ± 0.3	24.5 ± 0.4
12	25.3 ± 0.5	26.3 ± 0.6	26.6 ± 0.6	25.5 ± 0.4	25.7 ± 0.4	25.1 ± 0.5
13	26.0 ± 0.6	26.3 ± 0.6	26.6 ± 0.5	25.9 ± 0.6	26.5 ± 0.4	25.4 ± 0.4

a/ Entries are mean ± standard error of ten animals.

TABLE 28

FEED CONSUMPTION (g/day) IN MALE MICE FED RDX

Test Week	Control[b]	Dose Group (mg/kg/day) 10	14	20	28	40
1	4.2 ± 0.1	4.8 ± 0.2[a]	4.6 ± 0.1	4.7 ± 0.2	4.7 ± 0.1	4.8 ± 0.1
2	4.3 ± 0.1	4.9 ± 0.2	4.8 ± 0.1	4.9 ± 0.1[a]	4.6 ± 0.0	4.7 ± 0.1
3	4.4 ± 0.1	4.7 ± 0.2	4.7 ± 0.2	5.1 ± 0.2[a]	4.6 ± 0.1	4.7 ± 0.1
4	4.9 ± 0.2	5.3 ± 0.1	5.1 ± 0.1	5.4 ± 0.2[a]	4.7 ± 0.1	4.8 ± 0.1
5	4.6 ± 0.1	4.8 ± 0.2	5.1 ± 0.2	5.7 ± 0.2[a]	5.2 ± 0.1	5.3 ± 0.1
6	5.5 ± 0.2	5.5 ± 0.3	5.5 ± 0.3	5.9 ± 0.4	6.0 ± 0.3	5.8 ± 0.3
7	5.7 ± 0.5	6.0 ± 0.6	6.3 ± 0.7	5.8 ± 0.2	5.6 ± 0.2	5.4 ± 0.2
8	4.8 ± 0.2	5.1 ± 0.2	5.1 ± 0.2	5.0 ± 0.2	5.3 ± 0.2	5.0 ± 0.1
9	5.0 ± 0.1	5.2 ± 0.2	5.3 ± 0.2	5.3 ± 0.1	5.2 ± 0.3	5.3 ± 0.2
10	4.9 ± 0.2	5.2 ± 0.1	5.4 ± 0.2	5.2 ± 0.1	5.2 ± 0.2 (9)	5.2 ± 0.2
11	4.9 ± 0.2	5.0 ± 0.2	4.9 ± 0.2	5.3 ± 0.2	5.0 ± 0.1 (9)	4.9 ± 0.2
12	6.1 ± 0.3	6.1 ± 0.1	6.1 ± 0.2	6.6 ± 0.1	6.0 ± 0.2 (9)	5.7 ± 0.3
13	5.1 ± 0.1	5.1 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	5.2 ± 0.2 (9)	5.4 ± 0.2

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.
b/ Entries are mean ± standard error of five cages.

TABLE 29

FEED CONSUMPTION (g/day) IN FEMALE MICE FED RDX

Test Week	Control[b/]	Dose Group (mg/kg/day) 10	14	20	28	40
1	3.7 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.2 ± 0.1[a/]	4.3 ± 0.1[a/]
2	4.0 ± 0.1	4.1 ± 0.1	4.6 ± 0.1	3.8 ± 0.3	4.7 ± 0.2	4.5 ± 0.1
3	3.9 ± 0.1	4.1 ± 0.2	4.3 ± 0.1	4.1 ± 0.1	4.5 ± 0.1[a/]	4.3 ± 0.1
4	4.5 ± 0.1	4.8 ± 0.2	5.2 ± 0.1	4.6 ± 0.1	5.0 ± 0.2	4.9 ± 0.2
5	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.0	4.4 ± 0.1	4.7 ± 0.1	4.9 ± 0.1
6	5.1 ± 0.2	5.1 ± 0.2	5.2 ± 0.1	5.0 ± 0.1	5.2 ± 0.2	5.2 ± 0.2
7	5.0 ± 0.1	4.8 ± 0.0	5.1 ± 0.1	4.8 ± 0.1	5.3 ± 0.1	5.2 ± 0.1
8	4.8 ± 0.1	4.7 ± 0.1	5.0 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	5.0 ± 0.2
9	4.7 ± 0.1	5.0 ± 0.0	5.0 ± 0.1	5.0 ± 0.1	4.9 ± 0.2	5.1 ± 0.2
10	4.8 ± 0.1	4.9 ± 0.0	5.1 ± 0.1	4.8 ± 0.2	4.7 ± 0.1	4.9 ± 0.2
11	4.5 ± 0.1	4.6 ± 0.2	4.6 ± 0.1	4.5 ± 0.2	4.5 ± 0.2	4.3 ± 0.3
12	5.4 ± 0.2	5.5 ± 0.2	5.4 ± 0.2	5.2 ± 0.2	5.3 ± 0.1	5.0 ± 0.4
13	4.8 ± 0.2	4.5 ± 0.2	4.6 ± 0.2	4.4 ± 0.2	4.3 ± 0.2	4.5 ± 0.3

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.
b/ Entries are mean ± standard error of five cages.

TABLE 30

CALCULATED RDX INTAKE (mg/kg/day) IN MALE MICE

Test Week	Control[a]	Dose Group (mg/kg/day) 10	14	20	28	40
1	0.0	9.8 ± 0.3	12.9 ± 0.5	19.0 ± 1.0	26.3 ± 0.8	38.8 ± 1.6
2	0.0	9.4 ± 0.4	12.9 ± 0.4	18.7 ± 0.6	25.1 ± 0.6	36.2 ± 1.1
3	0.0	8.8 ± 0.4	12.5 ± 0.5	19.0 ± 0.9	24.3 ± 0.7	34.9 ± 0.7
4	0.0	11.9 ± 0.4	14.4 ± 0.5	21.4 ± 0.6	26.4 ± 0.8	38.5 ± 1.2
5	0.0	8.9 ± 0.5	14.3 ± 0.5	20.3 ± 0.7	31.0 ± 0.9	44.2 ± 1.5
6	0.0	10.8 ± 0.7	14.6 ± 0.8	21.4 ± 1.4	30.7 ± 1.7	41.7 ± 2.2
7	0.0	11.4 ± 1.0	15.9 ± 1.5	20.9 ± 0.7	28.1 ± 0.9	38.9 ± 1.9
8	0.0	8.8 ± 0.3	12.0 ± 0.5	17.7 ± 0.8	25.3 ± 0.8	35.2 ± 1.5
9	0.0	9.0 ± 0.4	12.4 ± 0.6	19.7 ± 0.5	25.2 ± 1.1	37.2 ± 1.7
10	0.0	9.9 ± 0.3	14.1 ± 0.6	18.5 ± 0.5	27.8 ± 1.2 (9)	39.2 ± 1.9
11	0.0	9.7 ± 0.5	13.2 ± 0.6	19.1 ± 0.9	27.2 ± 0.7 (9)	37.0 ± 2.4
12	0.0	12.1 ± 0.3	17.4 ± 0.7	24.8 ± 0.7	32.2 ± 1.0 (9)	46.7 ± 3.1
13	0.0	10.0 ± 0.4	14.6 ± 0.6	19.6 ± 0.9	27.3 ± 1.0 (9)	43.1 ± 1.8
1-13[b]	0.0	10.0 ± 0.3	13.9 ± 0.4	20.0 ± 0.5	27.5 ± 0.7	39.4 ± 1.0

a/ Entries are mean ± standard error of 10 animals except as noted in parenthesis, based on nominal concentrations.

b/ Mean ± standard error of weekly means.

TABLE 31

CALCULATED RDX INTAKE (mg/kg/day) IN FEMALE MICE

Test Week	Control[a/]	Dose Group (mg/kg/day)				
		10	14	20	28	40
1	0	9.7 ± 0.3	13.5 ± 0.5	20.0 ± 0.3	29.1 ± 1.0	42.2 ± 1.1
2	0	9.6 ± 0.3	14.6 ± 0.3	18.2 ± 1.5	31.1 ± 1.0	43.2 ± 1.4
3	0	9.0 ± 0.4	13.5 ± 0.2	18.7 ± 0.3	28.2 ± 1.0	39.0 ± 1.2
4	0	12.0 ± 0.4	17.2 ± 0.4	22.0 ± 0.4	33.0 ± 1.2	45.6 ± 1.6
5	0	9.4 ± 0.2	12.3 ± 0.2	18.7 ± 0.4	25.9 ± 0.6	40.2 ± 1.4
6	0	11.0 ± 0.4	15.2 ± 0.5	21.0 ± 0.3	30.8 ± 1.0	43.8 ± 2.2
7	0	10.3 ± 0.2	14.7 ± 0.0	19.9 ± 0.3	31.0 ± 0.8	43.3 ± 1.1
8	0	9.0 ± 0.2	12.8 ± 0.2	18.9 ± 0.3	27.0 ± 0.7	40.0 ± 2.2
9	0	9.3 ± 0.2	12.7 ± 0.4	20.0 ± 0.5	26.6 ± 1.1	40.0 ± 2.1
10	0	10.1 ± 0.3	14.7 ± 0.4	19.4 ± 0.6	28.2 ± 0.7	41.0 ± 2.3
11	0	9.5 ± 0.2	13.3 ± 0.2	18.1 ± 1.1	27.3 ± 1.2	36.1 ± 2.6
12	0	11.7 ± 0.2	16.1 ± 0.2	21.7 ± 1.1	32.2 ± 0.6	45.1 ± 3.3
13	0	9.5 ± 0.3	13.5 ± 0.4	18.0 ± 0.5	24.9 ± 1.0	40.3 ± 2.6
1-13[b/]	0	10.0 ± 0.3	14.2 ± 0.4	19.6 ± 0.4	28.9 ± 0.4	41.5 ± 0.7

a/ Entries are mean ± standard error of 10 animals, based on nominal concentration.
b/ Mean ± standard error of weekly means.

TABLE 32

LABORATORY DATA OF MALE MICE AFTER FEEDING OF RDX FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
ERYTHROCYTES ($X10^6$ /MM^3)	7.17 ± .41	7.60 ± .23	7.60 ± .09
HEINZ BODIES.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES. %	1.45 ± .15	1.20 ± .09	1.33 ± .18
HEMATOCRIT. VOL. %	42.4 ± 2.2	45.2 ± .8	44.8 ± .4
HEMOGLOBIN. GM. %	15.6 ± .2	15.4 ± .2	15.5 ± .1
METHEMOGLOBIN.	0.0 ± 0.0	0.0 ± 0.0	.6 ± .4
MCV. CUBIC MICRONS	59.3 ± 1.2	59.6 ± 1.1	59.0 ± 1.1
MCHB. MICRO MICROGMS.	22.0 ± 1.1	20.2 ± .4	20.4 ± .3
MCHBC. GM %	37.2 ± 1.7	34.0 ± .2	34.6 ± .3
PLATELETS ($X10^5$ /MM^3)	2.2 ± .4	3.1 ± .7	2.3 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	1.4 ± .3	1.5 ± .3	1.7 ± .7
NEUTROPHILS. %	12.6 ± 3.6	10.8 ± 2.4	27.4 ± 5.9
LYMPHOCYTES. %	87.0 ± 3.4	88.2 ± 1.9	72.2 ± 6.2
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	0.0 ± 0.0	.2 ± .2	.4 ± .4
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	.4 ± .4	.8 ± .8	0.0 ± 0.0
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT. IU/L	43.6 ± 18.5	67.0 ± 13.1	60.4 ± 14.4
BUN. MG %	29.4 ± 3.4	28.0 ± 1.8	29.2 ± 1.8

ENTRIES ARE MEAN ± STANDARD ERROR

TABLE 33

LABORATORY DATA OF FEMALE MICE AFTER FEEDING OF RDX FOR 3 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: MG/KG/DAY	0 (C. 5)	28 (T. 5)	40 (T. 5)
ERYTHROCYTES ($X10^6$ $/MM^3$)	7.19 ± .22	6.59 ± .59	7.28 ± .30
HEINZ BODIES.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES. %	1.63 ± .30	1.67 ± .25	1.43 ± .28
HEMATOCRIT. VOL. %	42.8 ± 1.8	42.6 ± 2.5	44.8 ± .5
HEMOGLOBIN. GM. %	15.6 ± .2	15.5 ± .4	15.4 ± .2
METHEMOGLOBIN.	.6 ± .4	.3 ± .3	0.0 ± 0.0
MCV. CUBIC MICRONS	59.6 ± 2.1	65.4 ± 2.4	61.9 ± 2.3
MCHB. MICRO MICROGMS.	21.7 ± .5	24.2 ± 1.8	21.2 ± .7
MCHBC. GM %	36.6 ± 1.3	36.8 ± 1.8	34.3 ± .2
PLATELETS ($X10^5$ $/MM^3$)	4.1 ± .9	2.7 ± .5	6.0 ± 1.5
LEUKOCYTES ($X10^3$ $/MM^3$)	1.7 ± .4	1.7 ± .4	1.5 ± .1
NEUTROPHILS. %	12.2 ± 3.8	12.2 ± 4.6	13.4 ± 2.7
LYMPHOCYTES. %	87.2 ± 3.7	87.2 ± 4.4	85.6 ± 2.4
BANDS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	1.0 ± .4 a/
BASOPHILS. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES. %	.6 ± .4	.6 ± .4	0.0 ± 0.0
ATYPICAL. %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC. %	0.0 ± 0.0	.4 ± .4	0.0 ± 0.0
SGPT. IU/L	74.0 ± 40.4	56.6 ± 33.2	57.6 ± 13.9
BUN. MG %	26.0 ± 1.2	26.6 ± 2.7	33.0 ± 3.9

ENTRIES ARE MEAN ± STANDARD ERROR

a/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison procedure

TABLE 34

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED RDX FOR 90 DAYS[a]

Sex	Dose (mg/kg/day)	Terminal Body Weight (g)	Absolute Organ Weight (g) Brain	Heart	Liver	Kidney	Spleen	Gonad
Male	0	28.5 ± 0.6	0.45 ± 0.01	0.14 ± 0.00	1.27 ± 0.04	0.49 ± 0.01	0.061 ± 0.002	0.24 ± 0.01 (9)
	28	27.4 ± 1.0	0.46 ± 0.01	0.15 ± 0.01 (9)	1.20 ± 0.09	0.58 ± 0.10	0.072 ± 0.008	0.23 ± 0.01 (9)
	40	28.6 ± 0.6	0.46 ± 0.01	0.15 ± 0.00	1.21 ± 0.07	0.50 ± 0.02	0.069 ± 0.004	0.23 ± 0.01
Female	0	25.9 ± 0.5	0.46 ± 0.00	0.13 ± 0.00	1.22 ± 0.05	0.40 ± 0.01	0.099 ± 0.002	0.038 ± 0.005 (5)
	28	26.5 ± 0.4	0.48 ± 0.00	0.13 ± 0.00	1.17 ± 0.04	0.37 ± 0.01	0.097 ± 0.005	0.042 ± 0.007 (5)
	40	25.4 ± 0.4	0.47 ± 0.01	0.13 ± 0.01	1.21 ± 0.05	0.38 ± 0.01	0.091 ± 0.005	0.036 ± 0.004 (5)

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/100 g body weight) Brain	Heart	Liver	Kidney	Spleen	Gonad
Male	0	1.60 ± 0.03	0.51 ± 0.02	4.50 ± 0.20	1.70 ± 0.00	0.21 ± 0.01	0.83 ± 0.02 (9)
	28	1.70 ± 0.08	0.54 ± 0.02 (9)	4.30 ± 0.20	2.20 ± 0.40	0.26 ± 0.03	0.85 ± 0.03 (9)
	40	1.63 ± 0.03	0.51 ± 0.01	4.30 ± 0.20	1.70 ± 0.00	0.24 ± 0.01	0.82 ± 0.02
Female	0	1.80 ± 0.03	0.52 ± 0.01	4.72 ± 0.14	1.54 ± 0.04	0.38 ± 0.01	0.14 ± 0.02 (5)
	28	1.80 ± 0.04	0.50 ± 0.02	4.43 ± 0.17	1.41 ± 0.03[b]	0.37 ± 0.02	0.15 ± 0.03 (5)
	40	1.85 ± 0.04	0.52 ± 0.03	4.76 ± 0.19	1.49 ± 0.03	0.36 ± 0.02	0.14 ± 0.02 (5)

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/g brain weight) Heart	Liver	Kidney	Spleen	Gonad
Male	0	0.32 ± 0.02	2.80 ± 0.10	1.09 ± 0.02	0.13 ± 0.00	0.52 ± 0.01 (9)
	28	0.32 ± 0.02 (9)	2.61 ± 0.20	1.28 ± 0.24	0.16 ± 0.02	0.50 ± 0.01 (9)
	40	0.32 ± 0.01	2.61 ± 0.13	1.07 ± 0.03	0.15 ± 0.01	0.50 ± 0.01
Female	0	0.29 ± 0.01	2.63 ± 0.11	0.86 ± 0.03	0.21 ± 0.01	0.08 ± 0.01 (5)
	28	0.28 ± 0.01	2.46 ± 0.09	0.78 ± 0.02	0.20 ± 0.01	0.09 ± 0.02 (5)
	40	0.28 ± 0.02	2.57 ± 0.09	0.81 ± 0.01	0.19 ± 0.01	0.08 ± 0.01 (5)

a/ Mean ± standard error for ten rats, except as noted in parenthesis.
b/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.

TABLE 35

BODY WEIGHTS (g) OF MALE MICE FED RDX (SUPPLEMENTAL STUDY)[a]

Test Week	Dose Group (mg/kg/day) 0	80	160[c]	320[d]
Initial	19.6 ± 0.4	19.2 ± 0.4	19.5 ± 0.3	19.5 ± 0.4
1	22.0 ± 0.2	21.9 ± 0.5	22.0 ± 0.2	21.7 ± 0.3
2	22.7 ± 0.3	22.9 ± 0.3	23.1 ± 0.3	22.9 ± 0.6
3	23.6 ± 0.3	24.0 ± 0.3	23.3 ± 0.3	23.1 ± 0.6
4	23.5 ± 0.3	23.8 ± 0.2	23.7 ± 0.3	23.9 ± 0.6
5	24.1 ± 0.3	24.0 ± 0.3	24.2 ± 0.2	24.2 ± 0.6
6	24.5 ± 0.3	24.9 ± 0.4	24.7 ± 0.3	25.3 ± 0.7
7	25.7 ± 0.4	24.9 ± 0.3	25.4 ± 0.2	26.1 ± 0.7
8	25.6 ± 0.3	25.6 ± 0.5	26.1 ± 0.3	26.7 ± 0.7
9	26.0 ± 0.3	26.1 ± 0.5	26.0 ± 0.3	27.3 ± 0.7
10	25.0 ± 0.4	26.0 ± 0.4	26.1 ± 0.4	27.3 ± 0.7[b]
11	26.7 ± 0.5	27.2 ± 0.4	27.2 ± 0.4	28.0 ± 1.2
12	25.7 ± 0.4	26.3 ± 0.5	26.2 ± 0.4	26.7 ± 1.0 (6)
13	26.5 ± 0.4	27.1 ± 0.4	27.1 ± 0.4	27.3 ± 1.1 (6)

a/ Entries are mean ± standard error of 10 animals except as noted in parenthesis.

b/ Significantly different from control, $p \leq 0.05$, Dunnett's multiple comparison.

c/ Received RDX at 60 mg/kg/day for first 2 weeks.

d/ Received RDX at 40 mg/kg/day for first 2 weeks.

TABLE 36

BODY WEIGHTS (g) OF FEMALE MICE FED RDX (SUPPLEMENTAL STUDY)[a/]

Test Week	Dose Group (mg/kg/day) 0	80	160 [c/]	320 [d/]
Initial	16.3 ± 0.3	16.6 ± 0.1	17.0 ± 0.3	16.5 ± 0.2
1	18.9 ± 0.3	19.4 ± 0.3	19.5 ± 0.4	19.7 ± 0.2
2	20.5 ± 0.2	20.6 ± 0.2	20.8 ± 0.6	20.3 ± 0.2
3	21.5 ± 0.3	21.1 ± 0.1	21.5 ± 0.3 (10)	20.8 ± 0.3
4	21.6 ± 0.3 (11)	21.7 ± 0.2	21.5 ± 0.2 (10)	21.5 ± 0.3
5	22.6 ± 0.7 (11)	21.7 ± 0.2	22.1 ± 0.2 (10)	21.9 ± 0.3
6	23.1 ± 0.3 (11)	22.8 ± 0.2	23.0 ± 0.3 (10)	23.1 ± 0.2 (11)
7	23.8 ± 0.3 (11)	23.7 ± 0.2	23.6 ± 0.2 (10)	23.9 ± 0.2 (11)
8	24.5 ± 0.4 (11)	24.2 ± 0.2	24.2 ± 0.2 (10)	25.1 ± 0.3 (11)
9	24.5 ± 0.4 (11)	23.7 ± 0.8	24.4 ± 0.2 (10)	25.3 ± 0.4 (11)
10	24.8 ± 0.5 (11)	24.3 ± 0.3	24.8 ± 0.3 (10)	25.8 ± 0.3 (10)
11	25.4 ± 0.5 (11)	25.8 ± 0.4	25.9 ± 0.4 (10)	26.9 ± 0.4 (10)
12	24.9 ± 0.4 (11)	24.8 ± 0.3	25.3 ± 0.4 (10)	26.1 ± 0.3 (10)
13	26.0 ± 0.4 (11)	25.8 ± 0.3	26.3 ± 0.4 (10)	27.5 ± 0.4 (10)[b/]

a/ Entries are mean ± standard error of 12 animals except as noted in parenthesis.

b/ Significantly different from control, $p \leq 0.05$, Dunnett's multiple comparison.

c/ Received RDX at 60 mg/kg/day for first 2 weeks.

d/ Received RDX at 46 mg/kg/day for first 2 weeks.

TABLE 37

FEED CONSUMPTION (g/day) IN MALE MICE FED RDX (SUPPLEMENTAL STUDY)[a]

Test Week	Dose Group (mg/kg/day) 0	80	160[b]	320[c]
1	5.4 ± 0.3	5.5 ± 0.2	5.8 ± 0.2	5.4 ± 0.2
2	4.8 ± 0.1	5.1 ± 0.3	5.0 ± 0.1	5.0 ± 0.1
3	5.4 ± 0.1	5.5 ± 0.2	5.5 ± 0.1	5.3 ± 0.2
4	5.7 ± 0.2	5.7 ± 0.2	5.4 ± 0.1	5.0 ± 0.1
5	5.4 ± 0.1	5.4 ± 0.2	5.5 ± 0.1	5.2 ± 0.2
6	5.2 ± 0.1	5.3 ± 0.2	5.3 ± 0.1	5.3 ± 0.2
7	5.2 ± 0.1	4.9 ± 0.1	5.1 ± 0.1	5.1 ± 0.1
8	5.0 ± 0.2	5.5 ± 0.3	5.1 ± 0.1	5.2 ± 0.2
9	5.1 ± 0.2	5.0 ± 0.2	5.3 ± 0.2	5.1 ± 0.2
10	5.1 ± 0.2	5.1 ± 0.2	5.2 ± 0.1	5.2 ± 0.1
11	5.0 ± 0.2	4.5 ± 0.1	7.0 ± 1.6	4.7 ± 0.2
12	4.6 ± 0.1	4.8 ± 0.2	5.7 ± 1.0	4.5 ± 0.2 (6)
13	6.5 ± 1.3	5.3 ± 0.1	5.6 ± 0.2	5.3 ± 0.2 (6)

a/ Entries are mean ± standard error of 10 animals except as noted in parenthesis.
b/ Received RDX at 60 mg/kg/day for first 2 weeks.
c/ Received RDX at 40 mg/kg/day for first 2 weeks.

TABLE 38

FEED CONSUMPTION (g/day) IN FEMALE MICE FED RDX (SUPPLEMENTAL STUDY)[a]

Test Week	Dose Group (mg/kg/day) 0	80	160[b]	320[c]
1	4.6 ± 0.2	4.9 ± 0.1	4.6 ± 0.2	4.8 ± 0.2
2	4.5 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
3	4.8 ± 0.2	5.1 ± 0.2	4.8 ± 0.1 (10)	4.8 ± 0.2
4	5.1 ± 0.2 (11)	5.2 ± 0.1	4.8 ± 0.1 (10)	5.0 ± 0.2
5	4.8 ± 0.2 (11)	4.8 ± 0.1	4.8 ± 0.1 (10)	5.0 ± 0.1
6	5.6 ± 0.5 (11)	4.8 ± 0.1	4.8 ± 0.1 (10)	4.8 ± 0.3 (11)
7	5.2 ± 0.2 (11)	4.8 ± 0.2	4.7 ± 0.1 (10)	5.3 ± 0.2 (11)
8	5.3 ± 0.2 (11)	5.0 ± 0.2	4.8 ± 0.1 (10)	5.5 ± 0.3 (11)
9	5.1 ± 0.2 (11)	4.9 ± 0.2	4.7 ± 0.1 (10)	5.4 ± 0.2 (11)
10	5.3 ± 0.3 (11)	5.0 ± 0.3	4.8 ± 0.1 (10)	5.3 ± 0.2 (10)
11	4.9 ± 0.2 (11)	4.7 ± 0.2	4.5 ± 0.1 (10)	4.6 ± 0.3 (10)
12	5.1 ± 0.2 (11)	5.0 ± 0.2	4.7 ± 0.2 (10)	4.8 ± 0.2 (10)
13	6.1 ± 0.6 (11)	5.5 ± 0.2	5.1 ± 0.2 (10)	5.6 ± 0.2 (10)

a/ Entries are mean ± standard error of 12 animals except as noted in parenthesis.

b/ Received RDX at 60 mg/kg/day for first 2 weeks.

c/ Received RDX at 40 mg/kg/day for first 2 weeks.

TABLE 39

MORTALITY OF MICE FED RDX FOR 90 DAYS (SUPPLEMENTAL STUDY)

Male		
Dose (mg/kg/day)	Deaths	Time of Death (Week)
0	0/10	
80	0/10	
160	0/10	
320	4/10	11, 11, 11, 11

Female		
Dose (mg/kg/day)	Deaths	Time of Death (Week)
0	0/11[a]	
80	0/12	
160	0/10[b]	
320	2/12	6, 11

a/ FMX62 missexed, taken off test week 4.

b/ FMX89 and FMX90 missexed, taken off test week 3.

TABLE 40

CALCULATED RDX INTAKE (mg/kg/day) IN MALE MICE (SUPPLEMENTAL STUDY)[a/]

Test Week	Dose Group (mg/kg/day) 0	80	160[b/]	320[c/]
1	0	106.0 ± 4.2	83.7 ± 2.8	53.6 ± 1.9
2	0	91.7 ± 3.9	70.0 ± 1.9	45.3 ± 1.6
3	0	69.0 ± 2.0	141.4 ± 3.7	278.8 ± 16.1
4	0	80.5 ± 2.9	164.0 ± 4.8	299.0 ± 9.5
5	0	77.3 ± 2.2	163.7 ± 3.5	299.4 ± 8.8
6	0	77.6 ± 2.4	154.5 ± 2.8	309.9 ± 11.6
7	0	70.7 ± 1.5	144.8 ± 3.7	284.5 ± 7.8
8	0	78.6 ± 4.4	140.8 ± 3.9	284.0 ± 6.7
9	0	75.8 ± 2.6	161.2 ± 4.7	286.4 ± 10.1
10	0	76.7 ± 2.3	157.9 ± 2.5	289.5 ± 7.3
11	0	68.0 ± 1.8	209.2 ± 46.1	273.6 ± 6.3
12	0	72.5 ± 2.6	172.9 ± 28.5	262.7 ± 12.8 (6)
13	0	89.8 ± 2.4	157.7 ± 4.5	370.4 ± 10.2 (6)
1-13[d/]	0	79.6 ± 3.0	147.8 ± 10.0	256.7 ± 26.5
3-13[d/]	0	76.0 ± 1.7	160.7 ± 5.7	294.4 ± 8.5

a/ Entries are mean ± standard error of 10 animals except as noted in parenthesis based on nominal concentration.
b/ For the first 2 weeks of the study, this group received 60 mg/kg/day.
c/ For the first 2 weeks of the study, this group received 40 mg/kg/day.
d/ Mean ± standard error of weekly means.

TABLE 41

CONCENTRATED RDX INTAKE (mg/kg/day) IN FEMALE MICE (SUPPLEMENTAL STUDY)[a/]

Test Week	Dose Group (mg/kg/day) 0	80	160[b/]	320[c/]
1	0	108.4 ± 2.4	75.1 ± 3.0	53.9 ± 2.1
2	0	91.8 ± 1.8	66.9 ± 1.7	45.7 ± 1.0
3	0	71.7 ± 2.4	134.7 ± 2.0 (10)	281.6 ± 8.3
4	0 (11)	82.4 ± 1.8	157.7 ± 5.0 (10)	327.4 ± 12.9
5	0 (11)	75.1 ± 1.4	155.1 ± 4.1 (10)	320.2 ± 8.3
6	0 (11)	78.0 ± 1.9	151.7 ± 4.3 (10)	307.2 ± 20.0 (11)
7	0 (11)	75.1 ± 2.6	143.3 ± 5.1 (10)	323.9 ± 14.2 (11)
8	0 (11)	75.6 ± 3.4	141.4 ± 2.9 (10)	324.6 ± 14.7 (11)
9	0 (11)	79.8 ± 2.6	151.3 ± 2.9 (10)	325.9 ± 13.4 (11)
10	0 (11)	82.0 ± 5.7	153.2 ± 1.6 (10)	312.9 ± 7.8 (10)
11	0 (11)	74.3 ± 3.3	143.7 ± 1.1 (10)	276.9 ± 16.7 (10)
12	0 (11)	78.6 ± 3.5	149.6 ± 5.7 (10)	292.3 ± 12.3 (10)
13	0 (11)	98.4 ± 3.3	148.2 ± 4.0 (10)	401.2 ± 22.1 (10)
1-13[d/]	0	82.4 ± 3.0	136.3 ± 8.2	276.4 ± 29.1
3-13[d/]	0	79.2 ± 2.2	148.1 ± 2.0	317.6 ± 10.0

a/ Entries are mean ± standard error of 12 animals except as noted in parenthesis based on nominal concentration.
b/ For the first 2 weeks of study, this group received 60 mg/kg/day.
c/ For the first 2 weeks of study, this group received 40 mg/kg/day.
d/ Mean ± standard error of weekly means.

TABLE 42

LABORATORY DATA OF MALE MICE AFTER FEEDING OF RDX FOR 3 MONTHS (SUPPLEMENTAL STUDY)

(C,N) CONTROL (T,N) TREATED N = NUMBER OF MICE

DOSE: MG/KG/DAY	0 (C, 5)	80 (T, 5)	160 (T, 5)[b/]	320 (T, 5)[c/]
ERYTHROCYTES ($X10^6/MM^3$)	7.78 ± .18	7.38 ± .18	6.84 ± .39[a/]	7.61 ± .09
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.22 ± .09	.78 ± .13	1.06 ± .18	1.40 ± .10
HEMATOCRIT, VOL. %	46.4 ± 1.5	45.8 ± 1.5	43.6 ± 1.7	46.2 ± .7
HEMOGLOBIN, GM. %	15.6 ± .3	15.5 ± .3	14.7 ± .3[a/]	15.3 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	59.6 ± .6	62.0 ± .8	64.1 ± 1.5[a/]	60.8 ± 1.4
MCHB, MICRO MICROGMS.	20.4 ± .2	21.0 ± .2	21.6 ± .9	20.1 ± .4
MCHBC, GM %	34.2 ± .6	33.9 ± .4	33.7 ± .7	33.1 ± .3
PLATELETS ($X10^5/MM^3$)	3.6 ± .5 (4)	4.8 ± 1.0	4.6 ± .6	4.4 ± .7
LEUKOCYTES ($X10^3/MM^3$)	3.3 ± .4	2.4 ± .4	2.9 ± .5	4.3 ± 1.2
NEUTROPHILS, %	12.2 ± 2.1	19.0 ± 3.1	16.0 ± 1.4	19.6 ± 3.1
LYMPHOCYTES, %	86.6 ± 2.4	80.8 ± 3.1	83.2 ± 1.5	78.4 ± 3.4
BANDS, %	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .4	.2 ± .2	.8 ± .5	2.0 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2
SGPT, IU/L	41.8 ± 7.4 (4)	45.0 ± 6.7	25.0 ± 2.6	28.8 ± 2.4
BUN, MG %	32.0 ± 4.0)	27.4 ± .5	29.4 ± 1.7	29.8 ± 2.7 (4)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP, $P < 0.05$, DUNNETT'S MULTIPLE COMPARISON.
b/ RECEIVED RDX AT 60 MG/KG/DAY FOR FIRST 2 WEEKS.
c/ RECEIVED RDX AT 40 MG/KG/DAY FOR FIRST 2 weeks.

TABLE 43

LABORATORY DATA OF FEMALE MICE AFTER FEEDING OF RDX FOR 3 MONTHS (SUPPLEMENTAL STUDY)

(C,N) CONTROL (T,N) TREATED N = NUMBER OF MICE

DOSE: MG/KG/DAY	0 (C, 4)	80 (T, 4)	160 (T, 5)[b]	320 (T, 5)[c]
ERYTHROCYTES ($X10^6/MM^3$)	7.69 ± .13	6.94 ± .22	7.63 ± .32	7.74 ± .22
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.68 ± .01	.82 ± .25 (2)	.85 ± .07	.55 ± .06
HEMATOCRIT, VOL. %	46.3 ± .8	42.5 ± 1.9	47.4 ± 1.5	46.8 ± .8
HEMOGLOBIN, GM. %	15.5 ± .4	14.8 ± .4	16.1 ± .6	15.6 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	60.2 ± .6	61.2 ± 1.8	62.3 ± 1.0	60.6 ± .7
MCHB, MICRO MICROGMS.	20.2 ± .1	21.4 ± .2[a]	21.2 ± .1[a]	20.1 ± .1
MCHBC, GM %	33.5 ± .3	35.0 ± 1.0	34.0 ± .4	33.2 ± .2
PLATELETS ($X10^5/MM^3$)	3.3 ± .8	3.4 ± .7 (2)	3.6 ± .7	4.6 ± .4
LEUKOCYTES ($X10^3/MM^3$)	3.5 ± .6	2.9 ± .8	3.6 ± .2	3.4 ± .3
NEUTROPHILS, %	15.5 ± 2.4	14.5 ± 3.9	18.6 ± 2.5	18.2 ± 3.8
LYMPHOCYTES, %	83.5 ± 1.8	84.5 ± 3.7	80.0 ± 2.4	80.4 ± 4.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .6	1.0 ± .4	1.4 ± .2	1.4 ± .7
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2
SGPT, IU/L	46.8 ± 22.7	46.0 (1)	25.0 ± 2.2	37.8 ± 15.3
BUN, MG %	27.3 ± 2.4	32.3 ± 5.8 (3)	26.0 ± 2.5	24.3 ± 2.3 (4)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP, $P < 0.05$, DUNNETT'S MULTIPLE COMPARISON.
b/ RECEIVED RDX AT 60 MG/KG/DAY FOR FIRST 2 WEEKS.
c/ RECEIVED RDX AT 40 MG/KG/DAY FOR FIRST 2 WEEKS.

TABLE 44

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED RDX FOR 90 DAYS (SUPPLEMENTAL STUDY)[a/]

Sex	Dose (mg/kg/day)	Terminal Body Weight (g)	Absolute Organ Weight (g) Brain	Heart	Liver	Kidney	Spleen	Gonad
Male	0	26.8 ± 0.4	0.42 ± 0.01	0.13 ± 0.01	1.17 ± 0.05	0.46 ± 0.02	0.06 ± 0.00	0.24 ± 0.01
	80	27.1 ± 0.4	0.42 ± 0.01	0.13 ± 0.01	1.19 ± 0.06	0.50 ± 0.02	0.07 ± 0.00	0.25 ± 0.01
	160[c/]	27.1 ± 0.4	0.43 ± 0.01	0.13 ± 0.01	1.31 ± 0.06	0.51 ± 0.01	0.06 ± 0.00	0.23 ± 0.01
	320[d/]	27.2 ± 0.8 (9)	0.46 ± 0.02 (7)	0.14 ± 0.02 (7)	1.48 ± 0.12 (7)[b/]	0.52 ± 0.03 (7)	0.05 ± 0.01 (7)	0.22 ± 0.01 (7)
Female	0	26.0 ± 0.4 (11)	0.45 ± 0.01 (11)	0.12 ± 0.01 (11)	1.17 ± 0.06 (11)	0.40 ± 0.02 (11)	0.09 ± 0.01 (11)	0.04 ± 0.00 (11)
	80	25.8 ± 0.3 (12)	0.45 ± 0.01 (12)	0.12 ± 0.02 (12)	1.22 ± 0.06 (12)	0.38 ± 0.01 (12)	0.07 ± 0.01 (12)	0.04 ± 0.00 (12)
	160[c/]	26.3 ± 0.4	0.47 ± 0.00	0.12 ± 0.01	1.23 ± 0.06	0.39 ± 0.01	0.09 ± 0.01	0.04 ± 0.00
	320[d/]	26.8 ± 0.8 (11)	0.46 ± 0.02	0.13 ± 0.01	1.51 ± 0.04[b/]	0.40 ± 0.01	0.09 ± 0.00	0.05 ± 0.00

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/100 g body weight) Brain	Heart	Liver	Kidney	Spleen	Gonad
Male	0	1.58 ± 0.04	0.48 ± 0.03	4.40 ± 0.20	1.74 ± 0.06	0.20 ± 0.01	0.90 ± 0.03
	80	1.54 ± 0.02	0.48 ± 0.02	4.40 ± 0.20	1.82 ± 0.05	0.25 ± 0.02	0.91 ± 0.03
	160	1.60 ± 0.05	0.47 ± 0.04	4.80 ± 0.20	1.89 ± 0.04	0.21 ± 0.02	0.86 ± 0.04
	320	1.70 ± 0.10 (7)	0.51 ± 0.05 (7)	5.50 ± 0.50(7)[b/]	1.91 ± 0.09 (7)	0.20 ± 0.03 (7)	0.82 ± 0.04 (7)
Female	0	1.73 ± 0.05 (11)	0.47 ± 0.02 (11)	4.50 ± 0.20 (11)	1.54 ± 0.05 (11)	0.33 ± 0.03 (11)	0.16 ± 0.01 (11)
	80	1.73 ± 0.04 (12)	0.47 ± 0.04 (12)	4.70 ± 0.20 (12)	1.46 ± 0.02 (12)	0.29 ± 0.03 (12)	0.14 ± 0.01 (12)
	160	1.79 ± 0.04	0.46 ± 0.02	4.70 ± 0.20	1.48 ± 0.04	0.33 ± 0.03	0.15 ± 0.01
	320	1.66 ± 0.05	0.48 ± 0.05	5.50 ± 0.20[b/]	1.46 ± 0.03	0.32 ± 0.02	0.18 ± 0.01

Sex	Dose (mg/kg/day)	Relative Organ Weight (g/g brain weight) Heart	Liver	Kidney	Spleen	Gonad
Male	0	0.31 ± 0.02	2.79 ± 0.12	1.11 ± 0.05	0.13 ± 0.01	0.57 ± 0.02
	80	0.31 ± 0.01	2.86 ± 0.14	1.19 ± 0.04	0.16 ± 0.01	0.59 ± 0.02
	160	0.29 ± 0.02	3.08 ± 0.19	1.19 ± 0.04	0.13 ± 0.01	0.54 ± 0.02
	320	0.30 ± 0.02 (7)	3.24 ± 0.18 (7)	1.14 ± 0.08 (7)	0.12 ± 0.02 (7)	0.49 ± 0.03 (7)
Female	0	0.28 ± 0.02 (11)	2.62 ± 0.15 (11)	0.89 ± 0.04 (11)	0.19 ± 0.01 (11)	0.09 ± 0.01 (11)
	80	0.27 ± 0.02 (12)	2.75 ± 0.15 (12)	0.85 ± 0.03 (12)	0.17 ± 0.02 (12)	0.08 ± 0.01 (12)
	160	0.26 ± 0.01	2.62 ± 0.15	0.83 ± 0.03	0.18 ± 0.02	0.08 ± 0.01
	320	0.29 ± 0.03	3.35 ± 0.17[b/]	0.89 ± 0.03	0.20 ± 0.01	0.11 ± 0.01

a/ Mean ± standard error for 10 mice, except as noted in parentheses.
b/ Significantly different from control group, $p < 0.05$, Dunnett's multiple comparison.
c/ Received RDX at 60 mg/kg/day for first 2 weeks.
d/ Received RDX at 40 mg/kg/day for first 2 weeks.

TABLE 45

SUMMARY OF LESIONS IN MALE MICE FED RDX FOR 90 DAYS (SUPPLEMENTAL STUDY)

Lesions[a/] Dose (mg/kg/day):	0										320[d/]									
Mouse No.:	61	62	63	64	65	66	67	68	69	70	71	72	73[c/]	74	75[c/]	76	77	78	79[c/]	80[c/]
Adrenal																				
Focal subcapsular fibroplasia				1							1	1	Autolysis				1	1	1	b/
Lung																				
Perivascular or peribronchiolar mononuclear cells aggregation			1												1				1	
Heart																				
Focal myocardial degeneration											±	±				±		±		2
Liver																				
Hepatocellular vacuolization--Centrilobular	1					1														
Periportal		1	1				1				2			1	1	2	2			
Diffuse					1				1										1	
Microgranuloma								1		1					1					
Increased karyomegaly of hepatocytes											±				±	±	±		±	
Kidney																				
Fatty metamorphosis of tubular epithelium	1		1	1		1	1	1		1				1			1	1		
Foci of mononuclear cells				1																
Hydronephrosis		1																		1
Tubular nephrosis											1				1				1	±
Urinary Bladder																				
Foci of mononuclear cells in submucosa	1																			
Distended lumen															X					

a/ Severity of lesions: ± - minimal; 1 - mild; 2 - moderate; X - present.
b/ Tissue not available for evaluation.
c/ Animal died week 11.
d/ Received RDX at 40 mg/kg/day for first 2 weeks.

TABLE 46

SUMMARY OF LESIONS IN FEMALE MICE FED RDX FOR 90 DAYS (SUPPLEMENTAL STUDY)

Lesions[a/] Dose (mg/kg/day):	0											320[d/]											
Mouse No.:	61	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80[b/]	81	82	83[c/]	84
Adrenal																							
Focal subcapsular fibroplasia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1
Fat infiltration			1		1	1		1	1	1	1	1	1	1	1	1	1	1		1	1		1
Ectopic cortical tissue																				X			
Lung																						Autolysis	
Perivascular or peribronchiolar mononuclear cells aggregation							1					1					1						
Heart																							
Focal myocardial degeneration																±					±		
Liver																							
Hepatocellular vacuolization--																							
Centrilobular				1		1	1								1	1	1	1					
Diffuse	1																						1
Microgranulomas						1	1							1	1		1	1		1	1		1
Focal necrosis										1				1									
Spleen																							
Lymphoid depletion																1							
Scar formation																X							
Kidney																							
Hydronephrosis						1	1		1			1		1						1	1		
Tubular nephrosis																			1				
Uterus																							
Dilated lumen											1		1		1								1
Endometritis																		±					
Thymus																							
Foci of hemorrhage																	1						
Eye																							
Degeneration of lens																			2				

a/ Severity of lesions: ± - minimal; 1 - mild; 2 - moderate; X - present.
b/ Animal died week 4.
c/ Animal died week 11.
d/ Received RDX at 40 mg/kg/day for first 2 weeks.

TABLE 47

MUTAGENICITY EVALUATION OF RDX USING THE SALMONELLA/MICROSOME PLATE TEST[a/]

	TA-1535		TA-5537		TA-1538		TA-98		TA-100	
	I[c/]	II[d/]	I	II	I	II	I	II	I	II
Control	26	14	7	9	16	32	28	42	119	108
Positive Control[b/]		211[e/]		155[e/]		528[e/]		542[e/]		1,590[e/]
RDX in DMSO										
1 μg	20	18	6	8	23	28	41	36	112	120
10 μg	24	12	6	6	20	24	32	44	128	118
100 μg	22	15	4	16[f/]	16	30	30	34	129	113
300 μg	35	10	2	8	14	30	30	40	139	115
1,000 μg	24	12	4	10	15	35	30	32	112	135

a/ Modification of Ames et al., Mutation Res., 31:347 (1975).

b/ Positive control: TA-1535, Cyclophosphamide (200 μg in 0.1 ml DMSO per plate); TA-1537, TA-1538, TA-98 and TA-100, Benzo[a]pyrene (5 μg in 0.1 ml DMSO per plate).

c/ I - test run without metabolic activation.

d/ II - test run with metabolic activation.

e/ Classified as mutagenic, i.e., mutagenic index (treated/control) $\geq$ than 2.

f/ This value is considered marginal ($1.5 \leq$ M.I. ≤ 2.0) and was repated. The repeated value's M.I. was 1.3.

TABLE 48

NUMBER OF RATS IN RDX DOMINANT LETHAL STUDY

	RDX (mg/kg/day)			
	0	5	16	50
Number of Males				
Tested	22	22	22	19
Impregnating				
Four females	19	17	14	6
Three females	3	4	7	10
Two females	0	0	1	3
One female	0	0	0	0
No females	0	1	0	0
Number of Females				
Mated	88	88	88	76
Pregnant (%)	85(97)	80(91)	79(90)	60(79)

TABLE 49

FIRST WEEK MATING RESULTS FROM RDX DOMINANT LETHAL STUDY IN MALE RATS

	RDX (mg/kg/day) 0	5	16	50
Values/Dam				
Corpora lutea	15.7 ± 0.4	15.1 ± 0.3	15.2 ± 0.3	14.8 ± 0.5
Implants	13.9 ± 0.4	13.6 ± 0.4	13.7 ± 0.3	12.1 ± 0.9
Live embryos	12.5 ± 0.5	12.5 ± 0.4	12.9 ± 0.3	10.9 ± 1.1
Dead embryos	1.4 ± 0.3	1.0 ± 0.2	0.8 ± 0.2	1.2 ± 0.4
Indexes				
Implantation[a]	89 ± 2	91 ± 3	91 ± 2	80 ± 5
Viability[b]	90 ± 2	92 ± 2	94 ± 1	87 ± 5

a Ratio of implants to corpora lutea x 100.
b Ratio of live embryos to implants x 100.

TABLE 50

SECOND WEEK MATING RESULTS FROM RDX DOMINANT LETHAL STUDY IN MALE RATS

	RDX (mg/kg/day)			
	0	5	16	50
Values/Dam				
Corpora lutea	15.3 ± 0.3	14.7 ± 0.3	14.9 ± 0.3	14.6 ± 0.4
Implants	14.1 ± 0.3	14.2 ± 0.3	13.3 ± 0.3	14.1 ± 0.4
Live embryos	13.0 ± 0.5	13.2 ± 0.3	12.3 ± 0.4	12.6 ± 0.5
Dead embryos	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	1.4 ± 0.3
Indexes				
Implantation[a]	93 ± 2	97 ± 1	90 ± 2	96 ± 1
Viability[b]	92 ± 2	93 ± 2	90 ± 2	89 ± 3

a Ratio of implants to corpora lutea x 100.
b Ratio of live embryos to implants x 100.

TABLE 51

COMBINED FIRST AND SECOND WEEK MATING RESULTS FROM RDX DOMINANT LETHAL STUDY IN MALE RATS

	RDX (mg/kg/day)			
	0	5	16	50
Values/Dam				
Corpora lutea	15.5 ± 0.2	14.9 ± 0.2	15.0 ± 0.2	14.8 ± 0.3
Implants	13.9 ± 0.2	13.9 ± 0.2	13.5 ± 0.2	12.9 ± 0.5
Live embryos	12.7 ± 0.3	12.9 ± 0.2	12.5 ± 0.3	11.6 ± 0.6
Dead embryos	1.2 ± 0.2	1.0 ± 0.2	0.9 ± 0.1	1.3 ± 0.2
Indexes				
Implantation[a]	91 ± 1	94 ± 2	90 ± 2	87 ± 3
Viability[b]	91 ± 1	93 ± 1	92 ± 2	88 ± 3

a Ratio of implants to corpora lutea x 100.
b Ratio of live embryos to implants x 100.

TABLE 52

EFFECT OF HYDROXYUREA AND RDX ADMINISTRATION DURING GESTATION ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Hydroxyurea[a/] 350 mg/kg	RDX (mg/kg/day) 0	0.2	2.0	20
Females					
Treated	24	24	24	24	25
Pregnant (%)[b/]	20(83)	24(100)	[illegible](100)	23(96)	24(96)
Deaths (%)[c/]	0	0	0	0	6(25)[c/]
Pregnant Survivors	20	24	24	23	17
Day 6 weight (g)	267 ± 2[d/]	263 ± 3	265 ± 3	263 ± 3	261 ± 3
Body weight changes (g) on gestation days					
6-7	1 ± 2	4 ± 2	2 ± 1	3 ± 1	-16 ± 2[d/]
6-9	9 ± 1	9 ± 2	8 ± 1	10 ± 2	-28 ± 3[d/]
9-13	15 ± 2	16 ± 2	1? ± 1	16 ± 2	19 ± 4
Corrected[e/]	47 ± 3	46 ± 2	49 ± 4	50 ± 4	21 ± 5[d/]
Final weight (g)	360 ± 5	369 ± 4	374 ± 6	378 ± 5	324 ± 10[d/]
Food consumption (g/day) for gestational days					
6-9	27 ± 2	25 ± 1	25 ± 1	25 ± 1	10 ± 2[d/]
9-13	23 ± 2	25 ± 1	26 ± 1	26 ± 1	19 ± 1[d/]
13-19	28 ± 1	28 ± 1	27 ± 2	29 ± 1	26 ± 1
Liver weight (g)	135 ± 0.2	13.8 ± 0.3	13.5 ± 0.4	13.9 ± 0.3	12.3 ± 0.5[d/]
(g/kg body weight)	37.7 ± 0.8	37.3 ± 0.6	35.9 ± 0.5	36.7 ± 0.7	37.8 ± 0.6[d/]
Implants/dam	14.8 ± 0.4	14.8 ± 0.3	14.6 ± 0.3	14.7 ± 0.4	13.8 ± 0.5
% Viable fetuses	86.9 ± 4.6	93.2 ± 1.3	97.6 ± 1.2[d/]	94.9 ± 1.6	81.4 ± 7.7
% Dead fetuses	0	0.3 ± 0.3	0	0	0.4 ± 0.4
% Early resoprtions	6.7 ± 1.6	6.0 ± 1.0	2.5 ± 0.8[d/]	4.8 ± 1.4	15.3 ± 7.8
% Later resorptions	6.3 ± 4.4	0.5 ± 0.4	0.5 ± 0.3	0.3 ± 0.3	1.6 ± 0.9
Complete resorptions	0	0	0	0	2

TABLE 52 (concluded)

	Hydroxy Urea[a/]	RDX (mg/kg/day)			
	350	0	0.2	2.0	20
Live Litters	20	24	24	23	15
Fetuses/day	12.7 ± 0.7	13.8 ± 0.4	14.2 ± 0.3	14.0 ± 0.4	12.7 ± 0.6
Fetal weigth (g)	3.26 ± 0.11	3.63 ± 0.06	3.69 ± 0.08	3.73 ± 0.08	3.36 ± 0.12
% Males	47 ± 4	53 ± 3	47 ± 4	49 ± 3	47 ± 5

a/ Administered 350 mg/kg/day on gestational day 6; vehicle on days 7-12.
b/ Based on intrauterine evidence of conception.
c/ Rat No. 24 was accidentally killed.
d/ Significantly different from control (two sample rank test).[6]
e/ Day 20 weight - day 0 weight - weight of the reproductive tract and contents.

TABLE 53

EXTERNAL ANOMALIES DETECTED IN FETUSES FROM RATS FOLLOWING ADMINISTRATION OF RDX OR HYDROXYUREA

	Hydroxyurea	RDX (mg/kg/day)			
	350 mg/kg/day	0	0.2	2.0	20
External Anomalies					
Litter - affected total	14/20	4/24	3/24	2/23	5/15
Fetuses - affected total	[illegible]5/255	6/331	3/338	2/321	10/191
EYDF	71(11)a/	0(0)	0(0)	0(0)	0(0)
FCLF	17(5)a/	0(0)	0(0)	0(0)	0(0)
SNDF	22(10)a/	0(0)	0(0)	0(0)	0(0)
SNRD	17(10)a/	0(0)	0(0)	0(0)	0(0)
SNUP	1(1)	0(0)	0(0)	0(0)	0(0)
SNLJ	11(6)a/	0(0)	0(0)	0(0)	0(0)

Note: EYDF - eye bulges absent
FCLF - cleft palate
SNDF - snout abnormal
SNRD - reduced snout
SNUP - snout upturned
SNLJ - reduced lower jaw

a/ Statistically different from the control group ($p < 0.05$). Number in parentheses is number of litters affected.

TABLE 54

SOFT TISSUE ANOMALIES DETECTED IN FETUSES FROM RATS FOLLOWING ADMINISTRATION OF RDX OR HYDROXYUREA

	Hydroxyurea	RDX mg/kg/day			
	350 mg/kg/day	0	0.2	2.0	20
Litter - Affected/total	14/20	9/24	6/24	11/23	8/15
Fetuses - Affected/total	53/95	16/122	8/121	16/116	12/67
BHL	21(10)a/	0(0)	0(0)	0(0)	0(0)
BH3	16(9)a/	0(0)	0(0)	0(0)	0(0)
BCC	4(2)	0(0)	0(0)	0(0)	0(0)
BEX	6(3)	0(0)	0(0)	0(0)	0(0)
OSD	6(3)	0(0)	0(0)	0(0)	0(0)
ONEB	8(4)a/	0(0)	0(0)	0(0)	0(0)
OREB	6(3)a/	0(0)	0(0)	0(0)	0(0)
EM	38(11)a/	0(0)	0(0)	0(0)	0(0)
LBCL	3(2)	0(0)	0(0)	0(0)	0(0)

Note: BHL - hydrocephalus lateral
BH3 - hydrocephalus third
BCC - cephalocele/hydrocele
BEX - exencephaly
OSD - damed skull
LCL - cleft lip
LBCL - cleft lip bilateral
EM - micropthalamia
ONEB - no eye bulges
OREB - reduced eye bulges

a/ Statistically different from the control group ($p < 0.05$). Number in parentheses is number of litters affected.

TABLE 55

SKELETAL ANOMALIES DETECTED IN FETUSES FROM RATS FOLLOWING ADMINISTRATION OF RDX OR HYDROXYUREA

Skeletal Anomalies	Hydroxyurea 350 mg/kg/day	RDX mg/kg/day 0	RDX mg/kg/day 0.2	RDX mg/kg/day 2.0	RDX mg/kg/day 20
Litter - affected/total	19/20	24/24	23/24	23/23	15/15
Fetuses - affected/total	158/161	161/209	149/218	156/205	94/125
SKS	58(16)	42(15)	50(16)	65(17)	28(10)
SKM	13(5)a/	0(0)	0(0)	1(1)	0(0)
SKC	37(12)a/	13(7)	20(6)	18(7)	10(5)
AXA	158(19)a/	151(24)	129(23)	139(22)	88(13)
AXR	137(18)a/	6(4)	13(5)	4(3)	5(2)
AXV	114(19)a/	31(19)	49(15)	29(17)	27(12)
AXS	125(19)	142(24)	114(23)	130(21)	88(14)

Note: SKS - anomalies of skull
SKM - anomalies of snout and mandible
SKC - anomalies of cranium
AXA - axial skeleton anomalies
AXR - anomalies of rib
AXV - anomalies of vertebrae
AXS - anomalies of sternebrae

a/ Statistically different from the control group ($p < 0.05$). Number in parentheses is number of litters affected.

TABLE 56

EFFECT OF 6-AMINONICOTINAMIDE AND RDX ON MATERNAL WELFARE AND REPRODUCTION IN RABBITS

	Control	6-Amino-nicotinamide	RDX (mg/kg/day) 0.2	2.0	20.0
Pregnant survivors	11	10	11	11	12
Body weight (kg)					
Day 0	3.80 ± 0.08[a/]	3.91 ± 0.11	3.73 ± 0.11	3.65 ± 0.15	4.03 ± 0.10
7	3.90 ± 0.09	4.00 ± 0.11	3.86 ± 0.09	3.79 ± 0.15	4.11 ± 0.09
14	3.89 ± 0.08	3.91 ± 0.10	3.90 ± 0.10	3.81 ± 0.11	3.90 ± 0.10
21	3.90 ± 0.08	3.84 ± 0.13	3.94 ± 0.12	3.82 ± 0.12	4.01 ± 0.09
28	3.94 ± 0.08	3.92 ± 0.11	3.97 ± 0.12	3.86 ± 0.14	4.10 ± 0.09
Implants/dam	9.5 ± 0.7	10.3 ± 0.5	10.2 ± 0.6	9.4 ± 0.9	9.6 ± 0.5
Viable fetuses (%)	85 ± 6	57 ± 10	82 ± 8	77 ± 11	94 ± 3
Dead fetuses (%)	0 ± 0	1 ± 1	8 ± 7	0 ± 0	1 ± 1
Early resorptions (%)	6 ± 5	34 ± 12[b/]	5 ± 3	4 ± 2	1 ± 1
Late resorptions (%)	8 ± 3	9 ± 3	5 ± 4	3 ± 2	3 ± 3
Live litters	11	10	11	11	12
Fetuses/dam	8.0 ± 0.7	6.0 ± 1.3	9.0 ± 0.7	8.6 ± 0.8	9.0 ± 0.5
Fetal weight (g)	43.0 ± 1.9	33.8 ± 1.9[b/]	40.1 ± 2.1	42.0 ± 2.2	39.0 ± 1.6

a/ Mean ± standard error.
b/ Significantly different from control (Dunnett's test).

TABLE 57

GROSS ANOMALIES IN RABBITS TREATED DURING GESTATION WITH 6-AMINONICOTINAMIDE OR RDX

			RDX (mg/kg/day)		
Gross Anomalies	Control	6-Amino-nicotinamide	0.2	2.0	20.0
Fetuses examined	88 (11)a/	60 (8)	99 (11)	94 (11)	110 (12)
Hematoma	0 (0)b/	0 (0)	1 (1)	0 (0)	0 (0)
Body blue	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Cranium raised	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Red spot on skull	0 (0)	3 (1)	0 (0)	0 (0)	0 (0)
No head	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Cranium misshaped	0 (0)	7 (1)	0 (0)	0 (0)	1 (1)
Meningocele	0 (0)	4 (2)	0 (0)	0 (0)	1 (1)
Eye bulges reduced	0 (0)	41 (7)	0 (0)	0 (0)	0 (0)
Eye bulges misshaped	0 (0)	0 (0)	0 (0)	0 (0)	3 (1)
Eye bulges enlarged	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
Ear pinna reduced	0 (0)	4 (4)	0 (0)	0 (0)	0 (0)
Snout abnormal	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Lower jaw reduced	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Premaxilla inc. fused	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)
Cleft palate	0 (0)	21 (5)	0 (0)	0 (0)	0 (0)
Abdominal wall defect	0 (0)	2 (2)	0 (0)	0 (0)	1 (1)
Gastroschisis	0 (0)	2 (2)	0 (0)	0 (0)	1 (1)
Spina bifida	0 (0)	9 (4)	0 (0)	0 (0)	3 (2)
Appendicular reduction anomaly	0 (0)	29 (7)	0 (0)	0 (0)	2 (2)
Rear legs malformed	0 (0)	3 (2)	0 (0)	0 (0)	0 (0)
Brachydactyly	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Trunk reduced	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Hindquarters reduced	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)
Tail defects	0 (0)	12 (4)	0 (0)	0 (0)	2 (1)
Tail absent	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)
Tail short	0 (0)	12 (4)	0 (0)	0 (0)	1 (1)
Small fetus	1 (1)	0 (0)	2 (1)	3 (2)	3 (2)

a/ Number of fetuses (number of litters) examined.
b/ Number of fetuses (number of litters) with anomaly.

TABLE 58

SOFT TISSUE ANOMALIES IN RABBITS TREATED DURING GESTATION WITH 6-AMINONICOTINAMIDE OR RDX

Soft Tissue Anomalies	Control	6-Amino-nicotinamide	RDX (mg/kg/day) 0.2	2.0	20.0
Fetuses examined	39 (11)a/	27 (7)	46 (11)	44 (11)	52 (12)
Hydrocephalus, lateral	0 (0)b/	1 (1)	0 (0)	0 (0)	0 (0)
Hydrocephalus, lateral-slight	0 (0)	1 (1)	1 (1)	0 (0)	0 (0)
Hydrocephalus, 4th ventricle-slight	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Longitudinal fissure enlarged	0 (0)	4 (2)	0 (0)	0 (0)	0 (0)
Nasal passage occluded	0 (0)	2 (1)	2 (1)	0 (0)	0 (0)
Reduced eye bulges	0 (0)	17 (5)	0 (0)	0 (0)	0 (0)
No eyelids	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)
Micropthalmia	0 (0)	20 (6)	0 (0)	0 (0)	0 (0)
Retina misshaped	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Cleft lip	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)
Cleft lip bilateral	0 (0)	8 (4)	0 (0)	0 (0)	0 (0)
Cleft palate	0 (0)	8 (3)	1 (1)	2 (1)	2 (1)
Trachea occluded	5 (4)	1 (1)	5 (4)	6 (4)	8 (7)
Right sided aortic arch	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Right ventricle distended	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Diaphragmatic hernia	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Stomach distended	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Blood in stomach	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
No kidneys	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)
Urinary bladder distended	0 (0)	1 (1)	2 (2)	5 (2)	0 (0)
Urinary bladder small	1 (1)	0 (0)	1 (1)	0 (0)	0 (0)
Bladder vessels engorged	0 (0)	1 (1)	2 (1)	0 (0)	0 (0)
Small fetus	2 (2)	2 (2)	6 (3)	1 (1)	3 (1)
Domed skull	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Club foot	0 (0)	11 (4)	0 (0)	0 (0)	0 (0)
Front paws flexed	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)
No tail	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)

a/ Number of fetuses (number of litters) examined.
b/ Number of fetuses (number of litters) with anomaly.

TABLE 59

SKELETAL ANOMALIES IN RABBITS TREATED DURING GESTATION WITH 6-AMINONICOTINAMIDE OR RDX

Skeletal Anomalies	Control	6-Amino-nicotinamide	RDX (mg/kg/day) 0.2	2.0	20.0
Fetuses examined	49 (11)a/	33 (8)	53 (11)	50 (11)	58 (12)
Tympanic annulus absent	0 (0)b/	0 (0)	0 (0)	0 (0)	1 (1)
Premaxillary process inc. ossified	0 (0)	10 (5)	0 (0)	0 (0)	1 (1)
Mandibles shortened	0 (0)	3 (2)	0 (0)	0 (0)	0 (0)
Frontal fontanel enlarged	0 (0)	1 (1)	5 (2)	2 (2)	8 (2)
Interparietal unossified	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Interparietal inc. ossified	0 (0)	4 (2)	0 (0)	0 (0)	0 (0)
Supraoccipital inc. ossified	0 (0)	4 (2)	0 (0)	0 (0)	0 (0)
Exoccipital misplaced	0 (0)	5 (3)	0 (0)	0 (0)	0 (0)
Ribs extra	0 (0)	3 (3)	0 (0)	1 (1)	0 (0)
Ribs unossified	0 (0)	4 (1)	0 (0)	0 (0)	0 (0)
Ribs malfused	0 (0)	26 (8)	0 (0)	2 (2)	0 (0)
Centri ossified normally	44 (10)	11 (3)	53 (11)	47 (11)	54 (11)
Centri lobed	0 (0)	9 (3)	0 (0)	1 (1)	0 (0)
Centri split	0 (0)	9 (4)	0 (0)	2 (2)	0 (0)
Centri vertical fusion	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Hemi-centri	0 (0)	16 (5)	0 (0)	1 (1)	0 (0)
Hemi-vertebra	1 (1)	15 (5)	0 (0)	2 (2)	0 (0)
Vertical fusion of vertebrae	0 (0)	12 (6)	0 (0)	0 (0)	1 (1)
Sternebrae ossified normally	34 (10)	10 (5)	38 (11)	32 (11)	37 (11)
Sternebrae unossified	4 (4)	12 (6)	12 (7)	8 (4)	12 (6)
Sternebrae inc. ossified	4 (3)	5 (2)	3 (2)	4 (4)	5 (2)
Sternebrae lobed	0 (0)	1 (1)	0 (0)	3 (2)	0 (0)
Sternebrae malalignment	4 (3)	11 (5)	3 (3)	7 (4)	0 (0)
Femur reduced	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Tibia reduced	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
Fibula reduced	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)

a/ Number of fetuses (number of litters) examined.
b/ Number of fetuses (number of litters) with anomaly.

TABLE 60

CALCULATED DOSES OF RDX CONSUMED BY F_0 MALE RATS

Test Week	Nominal RDX Dose (mg/kg/day) 5	16	50
1	4.01 ± 0.04[a]	12.9 ± 0.2	32.9 ± 0.6
2	3.73 ± 0.08	11.6 ± 0.2	35.0 ± 0.5
3	3.78 ± 0.05	12.7 ± 0.3	24.1 ± 0.3
4	4.18 ± 0.05	13.6 ± 0.2	38.0 ± 0.5
5	4.13 ± 0.05	13.4 ± 0.2	39.5 ± 0.5
6	4.11 ± 0.05	14.9 ± 1.6	45.9 ± 0.5
7	4.15 ± 0.05	13.4 ± 0.2	44.7 ± 0.5
8	4.31 ± 0.05	12.3 ± 0.1	42.2 ± 0.5
9	4.51 ± 0.05	14.5 ± 0.3	43.3 ± 0.6
10	4.80 ± 0.08	14.9 ± 0.4	46.8 ± 0.6
11	4.79 ± 0.05	15.3 ± 0.1	52.0 ± 1.8
12	4.73 ± 0.06	15.4 ± 0.2	50.7 ± 1.8
13[b]	4.71 ± 0.05	14.8 ± 0.2	41.5 ± 2.1
Average	4.3	13.8	41.3
SE	0.1	0.3	2.1

a Mean ± SE during the indicated week, based on nominal concentration.
b These males were later used in the dominant lethal mutation study.

TABLE 61

CALCULATED DOSES OF RDX CONSUMED BY F_0 FEMALE RATS

Test Week	Nominal RDX Dose (mg/kg/day) 5	16	50
1	4.02 ± 0.05[a]	12.5 ± 0.2	28.8 ± 0.8
2	3.46 ± 0.07	11.3 ± 0.1	37.2 ± 0.5
3	4.04 ± 0.05	12.8 ± 0.2	29.5 ± 0.3
4	4.59 ± 0.05	14.9 ± 0.3	40.4 ± 0.5
5	4.58 ± 0.06	14.8 ± 0.2	42.5 ± 0.9
6	4.92 ± 0.07	15.2 ± 0.2	49.2 ± 0.9
7	4.81 ± 0.08	15.5 ± 0.3	52.2 ± 0.6
8	4.20 ± 0.07	14.2 ± 0.2	45.5 ± 0.7
9	4.84 ± 0.21	14.5 ± 0.3	41.5 ± 1.7
10	4.53 ± 0.06	15.2 ± 0.3	38.8 ± 4.4
11	4.74 ± 0.08	15.9 ± 0.3	51.2 ± 1.0
12	5.07 ± 0.08	16.0 ± 0.2	58.4 ± 3.8
13	4.75 ± 0.10	15.2 ± 0.2	49.5 ± 0.9
Average	4.50	14.5	43.4
SE	0.13	0.4	2.4

a Mean ± SE during the indicated week, based on nominal concentration.

TABLE 62

CALCULATED DOSES OF RDX CONSUMED BY F1 MALE RATS

Test Week	Nominal RDX Dose (mg/kg/day) 5	16	50
21	12.48 ± 0.45[a]	38.9 ± 0.9	ND[b]
22	10.25 ± 0.18	31.1 ± 0.4	80.6
23	5.98 ± 0.06	19.0 ± 0.5	81.4
24	4.15 ± 0.47	17.0 ± 1.4	58.4
25	4.29 ± 0.46	14.0 ± 0.1	75.5
26	4.73 ± 0.06	14.0 ± 0.4	59.7
27	4.29 ± 0.04	12.3 ± 0.1	72.8
28	4.04 ± 0.04	14.0 ± 0.1	53.6
29	4.24 ± 0.05	13.2 ± 0.1	56.0
30	3.82 ± 0.05	13.0 ± 0.1	52.0
31	4.36 ± 0.06	13.9 ± 0.2	49.5
32	4.71 ± 0.06	15.0 ± 0.1	48.7
33	4.64 ± 0.08	14.0 ± 0.1	46.9
Average	5.54	17.6	61.3
SE	0.74	2.2	3.7

a Mean ± SE for indicated week or mean of four males from one litter based on nominal concentration.

b Not determined.

TABLE 63

CALCULATED DOSES OF RDX CONSUMED BY F_1 FEMALE RATS

Test Week	Nominal RDX Dose (mg/kg/day) 5	16	50
21	12.20 ± 0.32[a]	37.7 ± 1.8	ND[b]
22	9.79 ± 0.14	31.6 ± 3.3	93.1
23	5.79 ± 0.23	19.6 ± 1.6	90.8
24	5.47 ± 0.08	18.5 ± 1.6	63.1
25	4.05 ± 0.28	14.3 ± 1.2	84.9
26	4.97 ± 0.10	15.2 ± 1.1	67.0
27	4.61 ± 0.07	13.1 ± 0.3	81.9
28	4.66 ± 0.08	15.2 ± 0.8	58.2
29	4.86 ± 0.08	14.9 ± 0.3	66.2
30	4.58 ± 0.08	14.9 ± 0.3	61.1
31	5.18 ± 0.09	16.1 ± 0.4	56.8
32	5.38 ± 0.09	16.9 ± 0.4	56.2
33	4.68 ± 0.07	16.6 ± 0.3	56.1
Average	5.86	18.8	69.6
SE	0.66	2.0	4.1

a Mean ± SE during the indicated week or mean of two females from one litter based on nominal concentration.

b Not determined.

TABLE 64

TOTAL NUMBER OF MALE AND FEMALE RATS IN A TWO-GENERATION STUDY WITH RDX

	Nominal RDX Dose (mg/kg/day)			
	0	5	16	50
F_0 Generation				
Tested	44	44	44	44
Dead (%)	0 (0)	0 (0)	0 (0)	8 (18)[b,c]
F_1 Generation				
Liveborn	199	290	255	76
Stillborn (%)	8 (4)	6 (2)	4 (2)	16 (17)[b]
Weaned	173	277	229	6
Tested	52	52	52	6
Dead (%)	0 (0)	1 (2)	2 (4)	0 (0)
F_2 Generation				
Liveborn	282	284	248	22
Stillborn (%)	6 (2)	6 (2)	2 (1)	24 (52)[b]
Weaned	223	244	197	0[d]
Necropsied	20	20	20	0

a The males were also used in the dominant lethal mutation study.
b Significantly different from control (Fisher's exact probability test).
c Deaths included two males and six females.

TABLE 65

BODY WEIGHT (GM) OF F_0 MALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	16	50
0	216 ± 5[a]	219 ± 4	221 ± 3	214 ± 3
1	276 ± 5	279 ± 5	283 ± 4	261 ± 4[b]
2	308 ± 6	312 ± 5	308 ± 4	283 ± 4[b]
3	350 ± 7	351 ± 6	354 ± 4	326 ± 5[b]
4	375 ± 8	380 ± 6	383 ± 4	339 ± 5[b]
5	405 ± 9	410 ± 7	409 ± 5	363 ± 6[b]
6	431 ± 9	435 ± 8	435 ± 6	382 ± 6[b]
7	454 ± 10	457 ± 8	456 ± 7	403 ± 7[b]
8	473 ± 10	474 ± 9	475 ± 7	412 ± 7[b]
9	487 ± 11	487 ± 10	486 ± 7	422 ± 8[b]
10	501 ± 12	506 ± 11	497 ± 8	429 ± 7[b]
11	514 ± 12	519 ± 11	510 ± 8	444 ± 8[b]
12	528 ± 12	527 ± 10	524 ± 8	447 ± 8[b]
13[c]	530 ± 12	534 ± 12	528 ± 9	453 ± 9[b]

a Mean ± SE.

b Significantly different from control (Tukey's omega procedure).

c After the mating for this study, these males were then used in the dominant lethal mutation study.

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TABLE 66

BODY WEIGHT (GM) OF F_0 FEMALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	16	50
0	163 ± 2[a]	162 ± 2	160 ± 2	159 ± 3
1	192 ± 3	194 ± 3	190 ± 3	173 ± 3[b]
2	201 ± 3	202 ± 3	199 ± 3	184 ± 3[b]
3	219 ± 4	221 ± 4	218 ± 3	204 ± 3[b]
4	229 ± 4	229 ± 4	226 ± 3	206 ± 4[b]
5	238 ± 4	239 ± 4	237 ± 3	217 ± 4[b]
6	250 ± 4	250 ± 5	247 ± 3	224 ± 4[b]
7	258 ± 4	257 ± 4	255 ± 3	236 ± 4[b]
8	262 ± 5	261 ± 4	259 ± 3	237 ± 5[b]
9	264 ± 5	265 ± 5	263 ± 3	248 ± 5
10	267 ± 5	280 ± 5	269 ± 3	245 ± 6[b]
11	278 ± 5	280 ± 5	276 ± 3	250 ± 4[b]
12	284 ± 5	282 ± 5	279 ± 3	254 1 5[b]
13	285 ± 5	285 ± 5	280 ± 3	262 ± 5[b]

a Mean ± SE.

b Significantly different from control (Tukey's omega procedure).

TABLE 67

BODY WEIGHT (GM) OF F_1 MALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	16	50
20	51 ± 2[a]	45 ± 2	45 ± 2	42[b]
21	95 ± 3	87 ± 3	86 ± 2	86
22	150 ± 4	140 ± 4	132 ± 3[c]	114
23	185 ± 5	175 ± 4	167 ± 3[c]	141
24	224 ± 6	212 ± 5	202 ± 4[c]	166
25	262 ± 6	255 ± 5	245 ± 4	216
26	298 ± 7	287 ± 6	275 ± 5[c]	246
27	329 ± 7	318 ± 6	308 ± 6	282
28	352 ± 7	344 ± 7	333 ± 6	309
29	371 ± 8	362 ± 7	351 ± 6	326
30	392 ± 9	381 ± 8	368 ± 6	348
31	404 ± 8	395 ± 8	380 ± 6	361
32	421 ± 9	413 ± 8	397 ± 7	384
33	435 ± 10	426 ± 8	408 ± 7	397

a Mean ± SE.
b Mean of four males from one litter.
c Significantly different from control (Tukey's omega procedure).

TABLE 68

BODY WEIGHT (GM) OF F_1 FEMALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	16	50
20	48 ± 2[a]	42 ± 2	41 ± 1	41[b,c]
21	96 ± 3	79 ± 2[c]	77 ± 2[c]	74[c]
22	129 ± 3	118 ± 3[c]	111 ± 3[c]	97[c]
23	147 ± 3	140 ± 3	132 ± 3[c]	117[c]
24	167 ± 4	159 ± 3	152 ± 3[c]	131[c]
25	183 ± 4	179 ± 3	173 ± 3	154[c]
26	197 ± 4	194 ± 4	188 ± 4	171[c]
27	209 ± 4	207 ± 4	201 ± 4	183[c]
28	221 ± 4	218 ± 4	212 ± 4	196[c]
29	229 ± 5	227 ± 4	220 ± 4	201[c]
30	237 ± 5	236 ± 4	227 ± 4	214[c]
31	245 ± 5	243 ± 4	234 ± 4	222[c]
32	255 ± 5	252 ± 4	242 ± 4	227[c]
33	258 ± 5	255 ± 4	247 ± 4	233[c]

a Mean ± SE.

b Mean of two females from one litter.

c Significantly different from control (Tukey's omega procedure).

TABLE 69

FEED CONSUMPTION (GM/RAT/DAY) OF F_0 MALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day)			
	0	5	16	50
1	25.9 ± 0.3[a]	26.3 ± 0.4	26.4 ± 0.3	20.2 ± 0.3[b]
2	29.0 ± 1.1	28.9 ± 0.6	27.8 ± 0.4	24.6 ± 0.3[b]
3	27.6 ± 0.6	27.9 ± 0.5	28.6 ± 0.8	26.4 ± 0.2
4	28.2 ± 0.7	28.5 ± 0.5	29.0 ± 0.6	23.4 ± 0.3[b]
5	28.4 ± 0.6	28.6 ± 0.5	29.1 ± 0.5	24.8 ± 0.3[b]
6	27.8 ± 0.7	28.6 ± 0.5	32.4 ± 3.3	24.9 ± 0.3[b]
7	28.8 ± 0.5	28.4 ± 0.5	28.9 ± 0.5	26.0 ± 0.5[b]
8	28.5 ± 1.0	27.3 ± 0.5	28.0 ± 0.5	23.9 ± 0.4[b]
9	27.8 ± 0.6	27.8 ± 0.5	28.6 ± 0.8	24.0 ± 0.3[b]
10	27.3 ± 0.6	27.6 ± 0.6	27.2 ± 0.7	23.0 ± 0.4[b]
11	28.0 ± 0.6	28.4 ± 0.5	28.6 ± 0.4	26.2 ± 0.8
12	27.8 ± 0.6	27.7 ± 0.6	27.7 ± 0.5	23.5 ± 1.1[b]
13[c]	28.4 ± 0.6	27.7 ± 0.5	27.8 ± 0.6	23.6 ± 1.3[b]

a Mean ± SE.

b Significantly different from control (Tukey's omega procedure).

c These males were later used in the dominant lethal mutation study.

TABLE 70

FEED CONSUMPTION (GM/RAT/DAY) OF F_0 FEMALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	10	50
1	18.8 ± 0.1[a]	18.8 ± 0.3	17.7 ± 0.2[b]	12.4 ± 0.4[b]
2	18.3 ± 1.8	18.0 ± 0.4	17.9 ± 0.2	17.3 ± 0.4
3	18.3 ± 0.4	19.0 ± 0.4	18.2 ± 0.2	17.6 ± 0.4
4	19.6 ± 0.5	19.3 ± 0.4	19.1 ± 0.3	15.3 ± 0.3[b]
5	19.6 ± 0.6	18.3 ± 0.3	18.7 ± 0.2	16.1 ± 0.5[b]
6	19.4 ± 0.3	19.8 ± 0.5	19.0 ± 0.2	15.8 ± 0.4[b]
7	19.0 ± 0.4	18.7 ± 0.3	18.8 ± 0.3	17.7 ± 0.4
8	18.1 ± 0.4	17.7 ± 0.4	17.7 ± 0.2	15.0 ± 0.3[b]
9	17.7 ± 0.4	18.9 ± 1.1	17.7 ± 0.3	15.6 ± 0.8
10	17.4 ± 1.0	17.5 ± 0.3	17.2 ± 0.2	12.4 ± 1.4[b]
11	19.4 ± 0.6	19.1 ± 0.3	18.5 ± 0.3	16.5 ± 0.4[b]
12	19.0 ± 0.3	18.7 ± 0.4	18.0 ± 0.2	15.3 ± 0.8[b]
13	18.8 ± 0.4	18.4 ± 0.4	18.1 ± 0.3	16.2 ± 0.3[b]

a Mean ± SE.
b Significantly different from control (Tukey's omega procedure).

TABLE 71

FEED CONSUMPTION (GM/RAT/DAY) OF F_1 MALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	16	50
21	11.5 ± 0.5[a]	10.6 ± 0.3	10.1 ± 0.5	ND[b]
22	16.1 ± 0.5	15.0 ± 0.4	13.6 ± 0.4[d]	11.5[c]
23	20.1 ± 0.5	18.8 ± 0.5	17.7 ± 0.7[d]	14.3
24	22.9 ± 0.7	15.7 ± 1.6[d]	19.3 ± 1.5	12.1
25	25.6 ± 0.6	24.4 ± 0.5	24.0 ± 0.5	20.3
26	27.6 ± 0.8	25.6 ± 0.5	24.2 ± 0.7[d]	18.3
27	27.1 ± 0.6	25.9 ± 0.5	25.6 ± 0.5	25.5
28	27.0 ± 0.6	26.7 ± 0.4	26.5 ± 0.5	20.6
29	25.9 ± 1.1	26.2 ± 0.5	25.5 ± 0.5	22.7
30	25.7 ± 0.5	25.3 ± 0.5	24.7 ± 0.4	22.4
31	26.6 ± 0.6	25.2 ± 0.6	24.3 ± 0.3[d]	22.2
32	26.1 ± 0.7	26.0 ± 0.4	25.3 ± 0.5	23.2
33	25.9 ± 0.6	25.2 ± 0.5	24.4 ± 0.5	22.9

a Mean ± SE.

b Not determined.

c Mean of four males from one litter.

d Significantly different from control (Tukey's omega procedure).

TABLE 72

FEED CONSUMPTION (GM/RAT/DAY) OF F_1 FEMALE RATS THAT CONSUMED DIETS CONTAINING RDX

Test Week	Nominal RDX Dose (mg/kg/day) 0	5	16	50
21	10.9 ± 0.4[a]	9.5 ± 0.3[b]	8.8 ± 0.3[b]	ND[c]
22	14.1 ± 0.4	12.4 ± 0.3	12.0 ± 1.3	11.2[d]
23	17.6 ± 0.4	14.9 ± 0.7	14.9 ± 1.2	13.2
24	17.9 ± 0.3	16.3 ± 0.3	16.4 ± 1.4	10.3
25	19.2 ± 0.6	16.7 ± 1.2	18.0 ± 1.5	16.3
26	19.2 ± 0.4	18.5 ± 0.3	18.4 ± 1.5	14.3
27	19.1 ± 0.5	18.5 ± 0.4	18.1 ± 0.5	18.7
28	19.1 ± 0.4	19.0 ± 0.4	18.4 ± 0.9	14.2
29	19.4 ± 0.4	18.9 ± 0.3	18.0 ± 0.5	16.6
30	18.8 ± 0.4	18.9 ± 0.4	17.4 ± 0.3[b]	16.3
31	19.7 ± 0.5	18.5 ± 0.3	17.4 ± 0.4[b]	15.7
32	19.0 ± 0.4	18.3 ± 0.4	17.3 ± 0.4[b]	15.9
33	18.1 ± 0.3	17.1 ± 0.8	17.4 ± 0.3	16.3

a Mean ± SE.
b Significantly different from control (Tukey's omega procedure).
c Not determined.
d Mean of two females from one litter.

TABLE 73

NUMBER OF ADULTS, LITTERS AND PUPS FROM MATING OF F_0 GENERATION

	Nominal RDX Dose (mg/kg/day)			
	0	5	16	50
Number of Males				
Tested	22	22	22	22
Cohoused	22	22	22	19
Mated	19	21	21	16
Fertile	17	21	20	11
Number of Females				
Tested	22	22	22	22
Cohoused	22	22	22	17
Mated	19	21	21	16
Pregnant	17	21	20	11
Number of Live Litters				
Day 0	17	21	19	10
Day 7	16	21	19	4[c]
Day 14	16	21	19	1[c]
Day 21	16	21	19	1[c]
Number of Pups/Litter				
Day 0	11.7 ± 1.0[a]	13.8 ± 0.5	12.8 ± 0.5	7.6 ± 1.4[b]
Day 7	11.3 ± 0.9	13.5 ± 0.5	12.2 ± 0.5	9.0 ± 0.7
Day 14	10.8 ± 0.9	13.3 ± 0.5	11.6 ± 0.6	6.0 ± 0.0
Day 21	10.8 ± 0.9	13.2 ± 0.5[b]	11.5 ± 0.6	6.0 ± 0.0

a Mean ± SE.

b Significantly different from control (Tukey's omega procedure).

c Significantly different from control (Fisher's exact probability test).[2]

TABLE 74

GESTATIONAL BODY WEIGHT OF DAMS AND LACTATIONAL BODY WEIGHT OF PUPS IN FIRST MATING OF F_0 GENERATION

	Nominal RDX Dose (mg/kg/day)			
	0	5	16	50
Dam Body Weight (gm)				
Day 0	289 ± 6[a]	287 ± 5	290 ± 6	267 ± 7
Day 13	342 ± 7	339 ± 5	339 ± 6	304 ± 9[b]
Day 20	413 ± 11	410 ± 6	403 ± 6	337 ± 11[b]
Pup Body Weight (gm)				
Day 0	6.6 ± 0.2	6.3 ± 0.1	6.5 ± 0.2	6.2 ± 0.2
Day 4	8.6 ± 0.4	8.5 ± 0.2	8.2 ± 0.2	7.2 ± 0.1
Day 25	53 ± 2	47 ± 2	43 ± 2[b]	41 ± 0[b]

a Mean ± SE.
b Significantly different from control (Tukey's omega procedure).

TABLE 75

RESULTS FROM SECOND MATING OF THE F_0 GENERATION CONTROL AND HIGH DOSE FEMALES MATED WITH NONTREATED PROVEN MALE BREEDERS

	Nominal RDX Dose (mg/kg/day) 0	50
Number of Females		
Cohoused	10	10
Mated	8	8
Pregnant	2	2
Dam Body Weight (gm)		
Gestational Day 0	289 ± 5[a]	277 ± 6
Gestational Day 13	340 ± 7	298 ± 8
Gestational Day 21	359 ± 19	311 ± 14
Live Pups/Litter		
Lactational Day 0	14 ± 2	10 ± 1
Lactational Day 4	14 ± 2	2 ± 0
Lactational Day 21	12 ± 1	2 ± 0
Pup Body Weight (gm)		
Lactational Day 0	7.0 ± 0.3	6.2 ± 0.2
Lactational Day 4	9.8 ± 0.2	6.2 ± 0.2
Lactational Day 21	36 ± 4	35 ± 2.5

a Mean ± SE.

TABLE 76

NUMBER OF ADULTS, LITTERS AND PUPS FROM MATING OF F_1 GENERATION

	Nominal RDX Dose (mg/kg/day)			
	0	5	16	50
Number of Males				
Tested	26	26	26	4
Cohoused	26	26	25	2
Mated	22	24	23	2
Fertile	22	23	20	2
Number of Females				
Tested	26	26	26	2
Cohoused	26	25	23	2
Mated	23	24	23	2
Pregnant	22	23	20	2
Number of Live Litters				
Day 0	22	23	20	2
Day 7	22	23	19	0
Day 14	21	23	19	0
Day 21	21	23	18	0
Number of Pups/Litter				
Day 0	12.8 ± 0.6[a]	12.3 ± 0.6	12.4 ± 0.8	5.5 ± 1.5[b]
Day 7	11.2 ± 0.7	11.8 ± 0.6	11.8 ± 0.7	-
Day 14	11.6 ± 0.6	11.5 ± 0.6	11.6 ± 0.7	-
Day 21	10.6 ± 0.6	10.6 ± 0.7	11.0 ± 0.7	-

a Mean ± SE.
b Significantly different from control (Tukey's omega procedure).

TABLE 77

GESTATIONAL BODY WEIGHT OF DAMS AND LACTATIONAL BODY WEIGHT OF PUPS IN MATING OF F_1 GENERATION

	Nominal RDX Dose (mg/kg/day)			
	0	5	16	50
Dam Body Weight (gm)				
Day 0	255 ± 6[a]	254 ± 5	242 ± 5	236 ± 12
Day 13	308 ± 6	302 ± 5	274 ± 7[b]	275 ± 11
Day 20	380 ± 6	368 ± 6	336 ± 9[b]	311 ± 3[b]
Pup Body Weight (gm)				
Day 0	6.2 ± 0.1	6.3 ± 0.1	6.1 ± 0.1	7.4 ± 2.0
Day 4	7.9 ± 0.3	8.3 ± 0.3	7.4 ± 0.2	-
Day 21	30 ± 1	31 ± 1	26 ± 1	-

a Mean ± SE.
b Significantly different from control (Tukey's omega procedure).

TABLE 78

SUMMARY INDEXES OF OBSERVATIONS DURING A TWO-GENERATION STUDY WITH RDX

		Nominal RDX Dose (mg/kg/day)			
F_0 Generation	Indexes[a]	0	5	16	50
Males	Mating	86	95	95	84
	Fertility	89	100	95	69
Females	Mating	86	95	95	94
	Fertility	89	100	95	69
Pups	Viability	95	99	97	36
	Lactation	93	96	93	21
F_1 Generation					
Males	Mating	85	92	92	100
	Fertility	100	96	87	100
Females	Mating	88	96	100	100
	Fertility	96	96	87	100
Pups	Viability	91	95	92	0
	Lactation	86	90	75	0

a Mating = Number mated/cohoused x 100
Fertility = Number fertile (pregnant)/mated x 100
Viability = Number pups day 4/day 0 x 100
Lactation = Number pups day 21/day 4 x 100

TABLE 79

BODY AND ORGAN WEIGHTS OF RATS SACRIFICED FOR HISTOPATHOLOGICAL EVALUATION IN RDX TWO-GENERATION STUDY

Weight (gm)	Normal RDX Dose (mg/kg/day) 0	5	16
	MALES		
Body	56 ± 3 (10)[a]	57 ± 5 (10)	47 ± 3 (10)
Brain	1.46 ± 0.03	1.43 ± 0.04	1.35 ± 0.04
Heart	0.31 ± 0.02	0.32 ± 0.02	0.29 ± 0.03
Liver	2.68 ± 0.16	2.86 ± 0.30	2.25 ± 0.16
Kidneys	0.67 ± 0.03	0.71 ± 0.05	0.59 ± 0.04
Spleen	0.26 ± 0.02	0.26 ± 0.03	0.21 ± 0.01[b]
Gonads	0.35 ± 0.02	0.36 ± 0.04	0.24 ± 0.03[b]
	FEMALES		
Body	55 ± 3 (10)	56 ± 2 (10)	46 ± 2 (10)[b]
Brain	1.42 ± 0.04	1.38 ± 0.03	1.37 ± 0.03
Heart	0.27 ± 0.02	0.31 ± 0.02	0.26 ± 0.02
Liver	2.60 ± 0.15	2.62 ± 0.13	2.25 ± 0.13[b]
Kidneys	0.68 ± 0.04	0.65 ± 0.02	0.54 ± 0.02[b]
Spleen	0.28 ± 0.02	0.24 ± 0.01	0.22 ± 0.01[b]
Gonads	0.08 ± 0.03	0.05 ± 0.01	0.05 ± 0.01

a Mean ± S.E. - the number of rats is in parentheses.
b Significantly different from control (Tukey's omega procedure).

TABLE 80

HISTOPATHOLOGICAL EVALUATION OF TISSUES FROM RATS IN TWO-GENERATION STUDY WITH RDX

	Nominal RDX Dose (mg/kg/day)		
	0	5	16
Renal Cortical Cysts			
Males	4/10 (40)[a]	4/10 (40)	8/10 (80)
Females	3/10 (30)	4/10 (40)	8/10 (80)

a Number affected/number examined (%).

APPENDIX I

ASSAY REPORTS

INTEROFFICE COMMUNICATION

MIDWEST RESEARCH INSTITUTE

April 30, 1979

To: Jan Minor

From: Dan Helton

Subject: Protocol for HPLC assay of RDX in Methylcellulose-Tween 80 Suspension

I. Assay Procedure

A. Sample Preparation

1. Sampling

Two sampling techniques have been used. In the first procedure the sample, usually ∿ 25 ml in volume, is shaken vigorously for 1-2 min then a 2.5 ml aliquot is immediately taken from the center of the sample. In the second procedure a 2.5 ml aliquot is removed while being stirred with a magnetic stir bar. The second technique is necessary when the RDX concentration is above 0.04% due to rapid settling of the sample after agitation has stopped. The sampling technique has now been standardized using the stirring procedure.

2. Dilution

Sample aliquots were diluted with acetone to give a concentration of ∿ 15 mg RDX/ml.

B. Standards Preparation

Bulk samples of RDX were diluted with acetone to give concentrations of 100 ± 20% of that expected in the unknown. Typical final dilutions were ∿ 15 mg/ml. The bulk RDX contains 10% water.

C. Chromatographic System

1. Instrument: Waters Associates with 254 nm detector
2. Column: 300 x 4 mm I.D., μPak C_{18}
3. Eluent: Methanol/1% acetic acid in water, 25/75, v/v
4. Flow: 2 ml/min
5. RDX Retention Volume: 7 ml with baseline resolution

D. Calculations

Calculations were based on peak height from a least squares curve fit of the standards data using a minimum of three data points.

II. Results

The assay results will be supplied as individual memos as required.

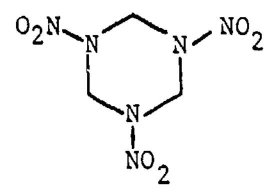
DH/ww

MIDWEST RESEARCH INSTITUTE
Project 4513-B
May 10, 1979

Data on: Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Submitted by: L. Wong

From: Danny O. Helton



$C_3H_6N_6O_6$

M.W. 222.13

Supplier: Holston Army Ammunition Plant (HSAAP)

Lot No.: HOL-435-37

I. Introduction

This sample was submitted for identification and assay, plus development of assay methods from mixtures of RDX with rat feed and aqueous suspensions. Discussed below are Identification and Assay of Lot HOL-435-37 (II), Assay of RDX from Rat Feed (III), and Assay of RDX from Aqueous Suspensions (IV), Stability of RDX on Rat Feed (V).

II. Identification and Assay of Lot HOL-435-37

A. Water Content

Duplicate 0.5 g samples were dried overnight to a constant weight under 0.1 mm Hg vacuum at room temperature. The observed weight loss was 2.2 ± 0.1%.

B. Identification of HMX and RDX

The presence of HMX and RDX was verified by high pressure liquid chromatography using the system described below.

1. Instrument: Waters Associates with 254 nm UV detector

2. Column: 300 x 4.0 mm I.D., μPak C_{18}

3. Eluant: methanol/water with 1% acetic acid, 25/75, v/v

4. Flow: 1 ml/min

5. Internal Standard: acetophenone

6. Results: By comparison of sample retention times with that of reference samples of RDX and HMX, the Lot contains only HMX and RDX as judged by absence of other peaks. The estimated detection limit for compounds having a similar chromaphore is < 0.5%. HMX eluted at 5 min, RDX at 7 min, and acetophenone at 13 min.

C. Conclusions

Weight loss on drying indicates a water content of 2.2 ± 0.1%. Examination by HPLC indicates HMX and RDX are the only organic components present assuming impurities would have a similar chromaphore. The estimated detection limit for impurities is < 0.5%.

III. Assay of RDX from Rat Feed

Although the sample is known to contain both RDX and HMX the results will be expressed as though only RDX were present. The results will be expressed on an anhydrous basis.

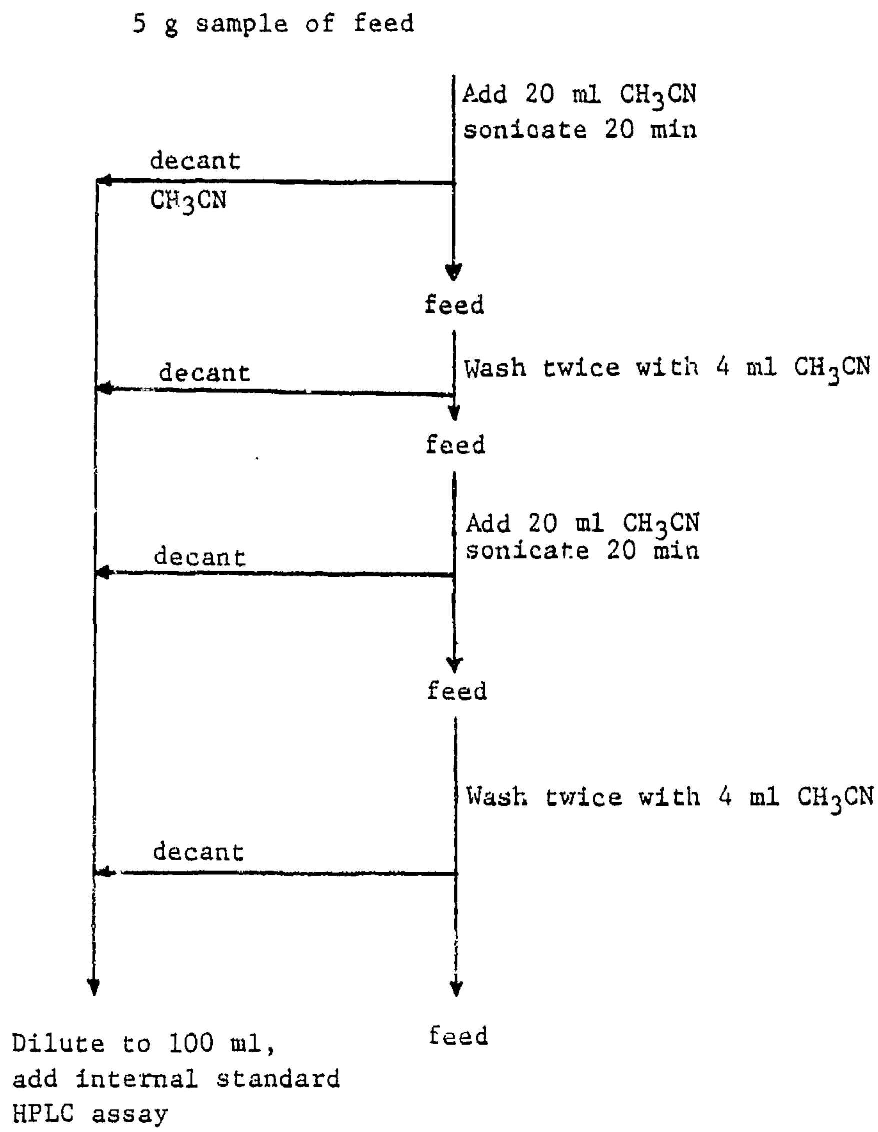
Determination of extraction efficiency was determined as indicated below.

A. Extraction Protocol

Feed samples were treated with acetonitrile containing RDX, allowed to dry, then reextracted with acetonitrile and assayed. A typical procedure is given below:

> Duplicate 25 g feed samples were treated with 25 ml CH_3CN containing 1 mg RDX/ml CH_3CN in a glass jar, shaken and allowed to air dry for 17 hr by placing near the front of a Class A hood having a face velocity of 125-150 CFM.

Duplicate samples were then extracted as outlined below.



B. Assay

The HPLC assay procedure described in II above was used. Calculations were based on peak height using the internal standard method. Standard concentrations of RDX-acetophenone were analyzed in triplicate using concentrations designed to be above, near, and below that expected in the sample. Concentrations in the unknowns were calculated from a least squares curve fit of the standards data.

C. Extraction Efficiency Results

The results from four nominal RDX concentrations are given below:

Extraction Efficiency of RDX from Rat Feed

Nominal RDX Concentration	Extraction Efficiency $\pm$ 1%[a]
10%	85
400 ppm (parts per million)	95
200 ppm	95
40 ppm	75

a/ The actual deviation values in all cases was less than or equal to 1%.

The assay results from actual unknowns are supplied in memo form as required.

IV. Assay of RDX from Aqueous Suspensions

Samples for this work were supplied in a methylcellulose-Tween 80 suspension.

A. Sample Preparation

1. Sampling

Two sampling techniques have been used. In the first procedure the sample, usually ∿ 25 ml in volume, is shaken vigorously for 1-2 min then a 2.5 ml aliquot is immediately taken from the center of the sample. In the second procedure a 2.5 ml aliquot is removed while being stirred with a magnetic stir bar. The second technique is necessary when the RDX concentration is above 0.04% due to rapid settling of the sample after agitation has stopped. The sampling technique has now been standardized using the stirring procedure.

2. Dilution

Sample aliquots were diluted with acetone to give a concentration of ∿ 15 mg RDX/ml.

B. Standards Preparation

Bulk samples of RDX were diluted with acetone to give concentrations of 100 $\pm$ 20% of that expected in the unknown. Typical final dilutions were ∿ 15 mg/ml.

C. Chromatographic System

See Section II above.

D. Calculations

Calculations were based on peak height from a least squares curve fit of the standards data using a minimum of three data points.

E. Results

Assay results from actual unknowns are supplied in memo form as required.

V. Stability of RDX on Rat Feed

Samples of RDX at the 40 parts per million (ppm) level were stored on rat feed at room temperature and 40°C. The RDX concentration was determined by HPLC as previously described in II above. The results were:

Storage at Room Temperature - 40 ppm RDX

	% Recovery RDX at Time of Storage[a/]		
	0 Days	7 Days	28 Days
Sample 1	75	72	73
Sample 2	75	74	70
Average	75	73	72

Storage at 40°C - 40 ppm RDX[a/]

	% Recovery RDX at Time of Storage[a/]		
	0 Days	7 Days	28 Days
Sample 1	75	65	57
Sample 2	75	65	60
Average	75	65	58

a/ Uncorrected for extraction efficiency

INTEROFFICE COMMUNICATION

MIDWEST RESEARCH INSTITUTE

August 7, 1979

To: Jan Minor

From: Dan Helton

Subject: Assays of RDX in Methylcellulose-Tween 80 Suspension

I. Introduction

The sample numbers are an internal code. Those having the same first number have the same nominal concentration. Those with the same letter designation (and first number) are from the same batch of suspension. The final digit designates sample number from a particular batch.

II. Assay Procedure

A. Assay Protocol

See memo of April 30, 1979.

B. Results

Individual assay results are listed in Table 1. Duplicates (see 2-C2 and 20-E4) agree well. More variation is seen between samples of the same lot (reflecting the irregularity of the suspensions), and between lots of the same nominal concentration (reflecting interbatch variation as well as suspension irregularity).

DH/HE/rj

TABLE 1

ASSAY RESULTS

Sample No.	Concentration (μg/ml) Nominal	Actual
0-A2	0	0.0
0-C1	0	0.0
0-C2	0	0.0
0-C3	0	1.1
0-D1	0	2
0-D2	0	2
1-A1	80	43.6
1-A2	80	28.9
1-B1	80	41.1
1-B2	80	44.0
1-B3	80	42.8
1-C1	80	401
1-C2	80	393
1-C3	80	381
1-D1	80	58.1
1-D2	80	26.7
1-D3	80	29.5
1-E1	80	46.5
1-E2	80	35.7
1-E3	80	34.2
1-F1	80	142
1-F2	80	133
1-F3	80	95
1-F4	80	190
1-F5	80	180
1-F6	80	130

Sample No.	Concentration (μg/ml) Nominal	Actual
2-C1	800	720
2-C2	800	610
2-C2*	800	600
2-C3	800	600
20-A1	8,000	2,980
20-2	8,000	3,100
20-B1	8,000	6,570
20-B2	8,000	6,430
20-B3	8,000	5,450
20-C1	8,000	5,580
20-C2	8,000	5,210
20-C3	8,000	5,460
20-D1	8,000	8,200
20-D2	8,000	8,590
20-D3	8,000	9,510
20-E1	8,000	10,600
20-E2	8,000	5,260
20-E3	8,000	10,400
20-E4	8,000	9,610
20-E4*	8,000	10,200
20-F1	8,000	6,390
20-F2	8,000	5,240

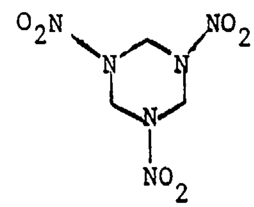
* Duplicate assay.

MIDWEST RESEARCH INSTITUTE
Project 4513-B
October 29, 1979

Data on: Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

To: Jim Cholakis

From: Danny O. Helton



$C_3H_6N_6O_6$

M. W. 222.13

Supplier: Holston Army Ammunition Plant (HSAAP)

Lot No.: HOL-435-37

I. Introduction

As requested in your memos of September 28 and October 4, samples of RDX/rat feed have been assayed for RDX content. Sample extraction efficiency was determined at five levels. Spiked samples are in storage to determine the long term stability of RDX on rat feed at room temperature.

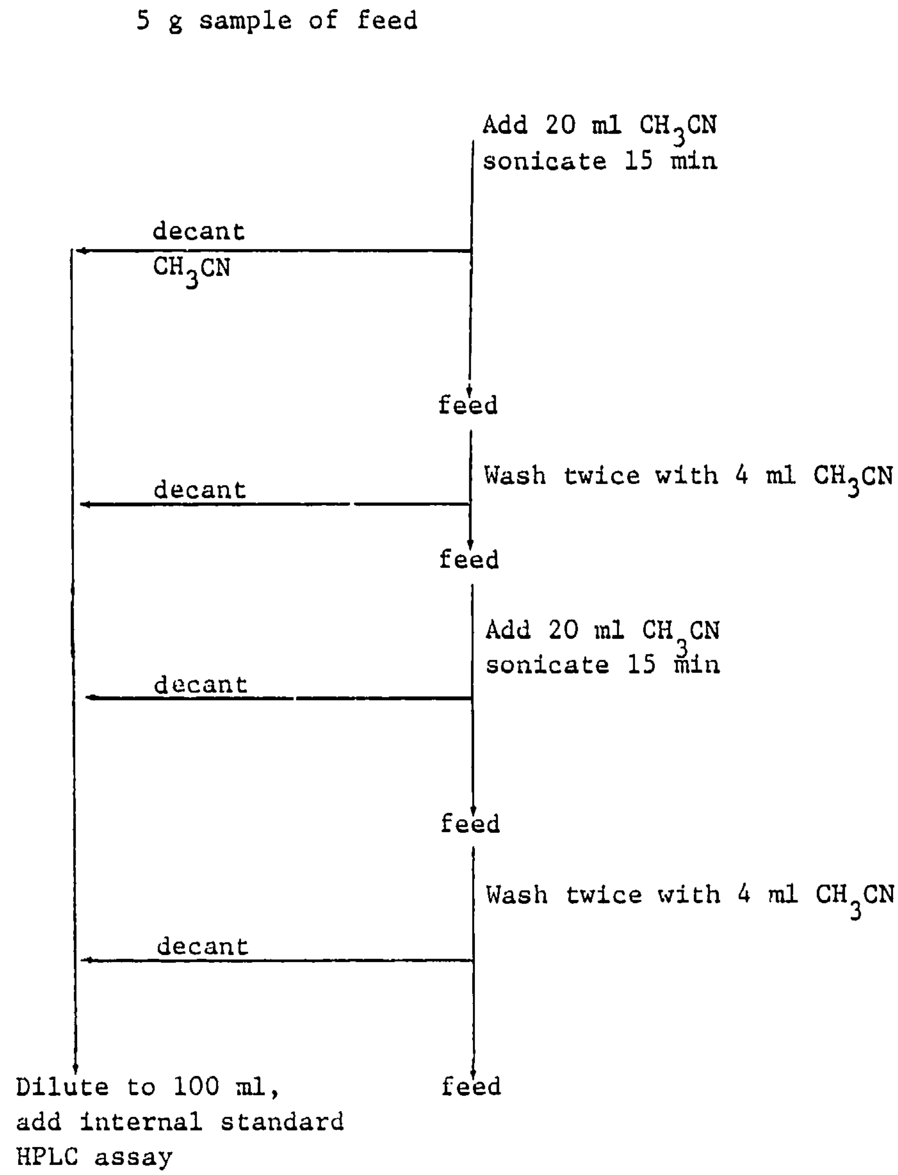
II. Assay of RDX from Rat Feed

Although the sample is known to contain both RDX and HMX, the results will be expressed as though only RDX were present. The results will be expressed on an anhydrous basis.

A. Extraction Protocol for Spiked Samples

Feed samples were treated with acetonitrile containing RDX, allowed to dry, then re-extracted with acetonitrile and assayed. A typical procedure is given below:

Duplicate 5 g feed samples were treated with 20 ml CH_3CN followed by sufficient RDX/CH_3CN to make nominal RDX/rat feed concentrations of 0.1, 0.2, 0.4, 0.8, and 1.6 mg RDX/g rat feed. After shaking, the samples were air dried by placing near the front of a Class A hood having a face velocity of 125-150 CFM. Samples were then extracted as outlined below.



Minor variations on the final dilution and addition of internal standard accommodated nominal RDX concentration ranges of 0.05 to 1.6 mg RDX/g feed.

B. Extraction Protocol for Unknowns

The procedure was the same as in A. above except RDX was not added to the samples.

C. Assay Procedure

1. Instrument: Altex 100 pump, Waters Associates U6K injector, Waters Associates 440 detector set at 254 nm

2. Column: Waters Associates μBondapak C_{18}, 300 x 4 mm ID

3. Eluent: CH_3OH/1% acetic acid in water, 50/50, v/v

4. Flow: 1 ml/min

5. Internal standard: Acetophenone at a concentration of 3.8×10^{-4} M

6. Sample concentration: 2.5 to 10 μg/ml

RDX had a retention time of 4.5 ml and acetophenone 6.0 ml. Calculations were based on peak height using the internal standard method. Standard concentrations of RDX-acetophenone were analyzed in triplicate before, during, and after sample injections on a daily basis. Concentrations in the unknowns were calculated from a least squares curve fit of the standards data.

D. Extraction Efficiency Results

The results from five RDX concentrations is given below:

Extraction Efficiency of RDX from Rat Feed

RDX Concentration in mg/g Feed	Extraction Efficiency
0.10	106 ± 4%[a/]
0.20	94 ± 2%[a/]
0.40	93 ± 2%[b/]
0.80	96 ± 2%[a/]
1.60	99 ± 1%[a/]

a/ Average of four determinations.
b/ Average of two determinations.

E. Assay Results for Samples

1. Samples from two generation reproductive study: All of these samples were stored at room temperature.

Date Prepared	Lot No.	Dose (mg/kg)	Nominal Conc. (mg/g feed)	Observed Conc. (mg/g feed)	Observed as % of Nominal
3/19/79	1	5	.0380	.054	142
	1	16	.1230	.137	112
	1	50	.3850	.570	148
5/16/79	8	5	.0780	.108	139
	8	16	.2447	.260	106
	8	50	.7502	.818	109
5/16/79	8	5	.0675	.081	121
	8	16	.2125	.234	110
	8	50	.6443	.708	110
6/26/79	14	5	.0955	.104	109
	14	16	.3018	.344	114
	14	50	.9497	.978	103
6/26/79	14	5	.0771	.096	125
	14	16	.2476	.260	105
	14	50	.7961	.621	78

2. Samples from 90-day subchronic study in rats:

Date Prepared	Lot No.	Dose (mg/kg)	Nominal Conc. (mg/g feed)	Observed Conc. (mg/g feed)	Observed as % of Nominal
10/16/78	1	10	.1000	.117	117
	1	14	.1400	.218	156
	1	20	.2000	.256	128
	1	28	.2800	.409	146
	1	40	.4000	.440	110
12/4/78	7	10	.1263	.235	186
	7	14	.1769	.265	150
	7	20	.2457	.346	141
	7	28	.3693	.539	146
	7	40	.4691	.727	155
12/22/78	10	10	.1478	.139	94
	10	14	.2113	.215	102
	10	20	.3030	.388	128
	10	28	.4213	.505	120
	10	40	.5917	.639	108

Lots 1 and 7 were stored at -10°C. Lot 10 was stored at room temperature.

3. Samples from 90-day subchronic study in mice:

Date Prepared	Lot No.	Dose (mg/kg)	Nominal Conc. (mg/g feed)	Observed Conc. (mg/g feed)	Observed as % of Nominal
10/10/78	1	10	.0500	.104	209
	1	14	.0700	.106	152
	1	20	.1000	.132	132
	1	28	.1400	.216	154
	1	40	.2000	.246	123
12/5/78	7	10	.0476	.087	182
	7	14	.0645	.090	140
	7	20	.0983	.128	130
	7	28	.1328	.191	144
	7	40	.1917	.299	156

Lots 1 and 7 were stored at -10°C.

4. Samples from supplemental 90-day subchronic study in mice:

Date Prepared	Lot No.	Dose (mg/kg)	Nominal Conc. (mg/g feed)	Observed Conc. (mg/g feed)	Observed as % of Nominal
1/15/79	2	80	.2929	.360	123
	2	160	.600	.648	108
	2	320	1.200	1.15	96
3/6/79	6	80	.400	.472	118
	6	160	.810	.956	117
	6	320	1.590	1.75	110

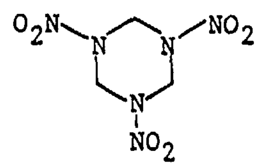
Lot 2 was stored at -10°C. Lot 6 was stored at room temperature.

MIDWEST RESEARCH INSTITUTE
Project No. 4513-B
January 7, 1980

Data on: Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Submitted by: Harry V. Ellis

From: Danny O. Helton



$C_3H_6N_6O_6$

M.W. 222.13

Supplier: Holston Army Ammunition Plant (HSAAP)

Lot No.: HOL-435-37

I. Introduction

An earlier report of May 10, 1979, indicated this lot contained $2.2 \pm 0.1\%$ water and RDX + HMX in an undetermined ratio. This report presents data on the RDX and HMX concentrations.

II. Assay for RDX

A. Experimental

1. Instrument: Pumps - Altex 100
   Programmer - Laboratory Data Control
   Injector - Waters Associates U6K
   Detector - Waters Associates 440
   Recorder - Heath-Schlumberger 255

2. Eluent: Methanol/H_2O, 50/50, v/v

3. Column: μBondapak C_{18}, 300 x 4 mm ID

4. Flow: 1 ml/min

5. Detector: UV at 254 nm

6. Internal standard: Acetophenone at a concentration of 3×10^{-4} M in CH_3CN.

7. Sample concentration: ∿ 0.6 mg/ml CH_3CN

8. Reference sample: Lot HOL475-1, I.D. No. PA150, was obtained from U.S. Army Armament R&D Command, Aberdeen Proving Ground, Edgewood, MD. Dr. Leslie Eng indicated this lot was a standard analytical reference material (SARM) of > 99% purity. For calculation purposes the sample was assumed to be 100% pure.

B. Results

RDX had a retention volume of 6 ml and acetophenone eluted at 9 ml. Based on peak height comparison to the reference sample, Lot HOL-435-37 contains 88.6 ± 0.9% RDX. Two weighings of the standard and three of the unknown were used.

The chromatogram of HOL-435-37 showed an additional peak at 4 ml retention volume having a peak area of ∿ 6% relative to the RDX peak at 100%. To examine the presence of minor components the eluent was changed to methanol/1% acetic acid in water, 30/70, v/v. Table 1 gives the retention times of several compounds using these new conditions.

TABLE 1

RETENTION VOLUME OF SELECTED NITRAMINES

Nitramine	Retention Volume, ml
SEX[a/]	5.0
HMX[b/]	6.7
TAX[c/]	7.0
RDX	11.5

a/ Octahydro-1-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine.
b/ Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.
c/ Hexahydro-1-acetyl-3,5-dinitro-1,3,5-triazine.

Lot HOL-435-37 gave peaks at 11.5 and 6.7 ml. Co-injection of Lot HOL-435-37 and HMX caused an increase in the 6.7 ml peak. Co-injection of Lot HOL-435-37 and TAX showed peaks at 6.7 and 7.0 ml. The concentration of SEX and TAX can only be approximated as < 0.5% since the purity of these reference samples is not well known.

By difference the HMX content is (100% - 2.2% water - 88.6% RDX) = ∿ 9%.

III. Conclusions

Lot HOL-435-37 contains 2.2 ± 0.1% water, 88.6 ± 0.9% RDX, and ∿ 9% HMX. No other components were detected by high performance liquid chromatography with an estimated detection limit of < 0.5%.

APPENDIX II

PROJECT PROTOCOL AND STANDARD OPERATING PROCEDURES
SALMONELLA/MICROSOME (AMES) ASSAY OF RDX

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MRI Project No. 4513-B

SALMONELLA/MICROSOME (AMES) ASSAY OF RDX

PROJECT PROTOCOL
AND
STANDARD OPERATING PROCEDURES

GENERAL PROTOCOL FOR THE SALMONELLA/MICROSOME (AMES) TEST TO SCREEN CHEMICALS FOR CARCINOGENIC POTENTIAL

I. INTRODUCTION

The Salmonella/microsome mutagenicity (Ames) test[1/] has shown a high correlation of detecting carcinogens as mutagens. In extensive testing, the Ames test has demonstrated a 90% (156/174)[2/] accuracy of detecting carcinogens as mutagens. In another series, less than 10% of the noncarcinogens tested were mutagenic.[2/] More recently, Purchase et al.,[3/] have reported an accuracy of 91% (53/58) for predicting carcinogens as mutagens, whereas the number of false positives was only 7% (4/62). The test is very sensitive and has been reported to be able to detect mutagens in nanogram quantities.[2/] Compounds are incubated with several specially constructed histidine auxotrophic mutants of *Salmonella typhimurium*, designed to detect base-pair substitution and frame-shift back mutations. If the compound causes a reversion in the histidine gene, then the mutant cells will grow in a histidine deficient medium and are readily scored. The compounds are assayed with and without an in vitro metabolic activation system to simulate mammalian metabolism.

II. MATERIALS AND METHODS

A. Bacteria and Culture Media

The bacteria used in the Ames test are the *Salmonella typhimurium* tester strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100. These tester strains are histidine auxotrophs and are used to detect base-pair substitutions (TA-1535 and TA-100) and frameshift reverse mutations (TA-1537, TA-1538 and TA-98). All tester strains were obtained from Dr. Bruce Ames, University of California, Berkeley.

The medium used in the mutagenesis assay is 1.5% Bacto-Difco Agar in Vogel-Bonner Medium E[4/] with 2% glucose. Difco Nutrient broth is used to prepare stock cultures.

B. <u>Testing of Bacterial Strains for Integrity</u>

All bacterial strains are tested routinely for histidine requirement and deep rough (rfa), which is a mutation in which the cell wall is deficient in lipopolysaccharide components, making it more permeable to large molecules every 4 months. Strains TA-98 and TA-100 are also checked for R factor every 4 months. All integrity tests are performed according to the procedures described by Ames et al.[1/]

1. <u>Histidine Requirement</u>: To test for histidine requirement, each culture (0.1 ml) is plated in a soft agar overlay (2 ml) on minimal plates with or without histidine (0.1 ml of 0.1 M L-histidine). Growth on the histidine difficient plate indicates that the bacteria have lost their dependence for histidine.

2. <u>Test for Deep rfa</u>: Strains having the rfa are tested for crystal violet (and/or deoxycholate) sensitivity. A sterile filter paper disc containing crystal violet (10 μl of 1 mg/ml or 2 mg deoxycholate) is placed on a nutrient agar petri dish containing 0.1 ml (about 10^8 bacteria)

of the nutrient broth culture to be tested in a thin overlay of agar (top agar). After 12 hr incubation at 37°C, a clear zone of inhibition around the disc (about 14 mm diameter) indicates the presence of the rfa mutation, which permits large molecules such as crystal violet to enter the bacteria and inhibit growth. Wild-type strains are not inhibited.

3. Testing for R factor: The tester strains with R factors (TA-100 and TA-98) are checked routinely for the presence of the ampicillin resistant R factor. Each culture (0.1 ml) is plated in a soft agar overlayer (2.0 ml) on nutrient agar plates. A disc containing 10 μg of ampicillin (Difco) is placed in the center of the plate and the plate incubated for 12 to 24 hr at 37°C. Strains which do not contain the R factor will show a zone of growth inhibition around the ampicillin disk, whereas R factor containing strains will not.

C. Preparation of Test Compounds

Test compounds are dissolved in dimethylsulfoxide (DMSO) at concentrations of 10 mg/ml, 3 mg/ml and 1 mg/ml. Lower doses (100 μg/ml and 10 μg/ml) are prepared by subsequent serial dilution with DMSO.

D. Preparation of Rat Liver S-9 Fraction

The S-9 fraction from rat liver is prepared according to the procedure of Ames et al.[1/] Young male Charles River rats weighing 180 to 200 g are given a single intraperitoneal injection of Aroclor 1254 at a dosage of 0.5 mg/g of body weight. The compound is prepared in corn oil at a concentration of 200 mg/ml. Five days after dosing, the rats are killed. The livers are aseptically removed and placed in a cold sterile petri dish containing 10.0 ml of 0.15 M KCl. The livers are swirled in the petri dish and removed with sterile forceps to a second petri dish containing 3.0 ml of 0.15 M KCl/g of liver. The liver is then minced with sterile scapel and scissors, transferred to a cold teflon in glass homogenizer and homogenized (25% w/v) at low speed (three to five strokes). The homogenates are transferred into sterile centrifuge tubes and centrifuged for 10 min at 9,000 x g. The supernatant (S-9 fraction) is decanted and 2 ml aliquots frozen in an ethanol-dry ice bath and stored at -80°C in a Revco freezer. Sufficient S-9 fraction is thawed each time and kept on ice just before use.

Prior to use, each new batch of S-9 fraction is tested for its efficiency for metabolic activation. For these tests, we use the TA-100 tester strain with 7,12-dimethylbenzanthracene (20 μg/plate) and TA-1538 with cyclophosphamide (200 μg/plate) as our reference carcinogens. Increasing amounts (0.1 ml, 0.2 ml ... 0.7 ml) of S-9 fraction are added to the complete test system and the cultures incubated as in the normal assay. Each assay is run against a batch of S-9 fraction previously determined to give satisfactory results with DMBA. The concentration of the S-9 fraction from the new batch is then adjusted to give results comparable to the reference batch.

E. Microsomal Activation System

The microsomal activation system used for the mutagenesis assay contained per ml: S-9 fraction (0.08 ml), NADP (4 μM), glucose-6-phosphate

(5 μM), KCl (33 μM), $MgCl_2$ (8 μM) and sodium phosphate buffer (100 μM), pH 7.4. The microsomal activation system is prepared fresh and maintained on ice before and during use.

F. Positive Control Chemicals

The following chemicals are used as positive controls to assure that (1) all strains are capable of mutation during a particular experiment, and (2) the metabolic activation system is working properly. All the chemicals that are used as positive controls have been reported to be mutagenic and require metabolic activation in the Ames' test.[2/]

Strain	Positive Control Chemical
TA-1535	Cyclophosphamide (CP)
TA-1537	Benzo[a]pyrene (BP) 7,12-Dimethylbenz[a]anthracene (DMBA)
TA-1538	BP, DMBA
TA-98	BP, DMBA
TA-100	DMBA, BP

G. Assay Procedure

The histidine requiring Salmonella typhimurium tester strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100 are exposed to selected concentrations of the test compound. The concentrations are 1,000, 300, 100, 10 and 1 μg/plate to insure a wide dosage range. For those compounds showing bactericidal activity, as indicated by a reduction in the number of spontaneous revertants in the treated plates relative to the control, additional concentrations of 0.1 and 0.01 μg/plate are used. The test compound is dissolved in DMSO as described above and run in duplicate with and without metabolic activation. To insure that large variations in pH will not interfere with cell growth, the pH of the soft agar overlay containing the highest drug concentration is measured and the pH adjusted to pH 6.5 to 7.0. The pH is measured using Fischer Scientific Short Range ALKACID® test paper. Each strain is run with a respective positive control. The cultures are incubated at 37°C for 48 hr. At the end of the incubation time macroscopic colonies are scored using a Darkfield Quebec Colony Counter. Background lawn is examined so that toxic effects of the drug can be determined.

H. Analysis of Results

The mutagen assay is scored as the ratio of the number of colonies (total revertants) in the experimental plates over the number of colonies in the control plates (spontaneous revertants). This is taken as the mutagenic index. A compound is generally considered to have a negative response if its mutagenic index is less than 2.0 for a concentration of 2.0 μM (about 500 μg/plate) or more per plate.[1/] The assay is repeated on all drugs showing a positive response (MI $\geq$ 2.0) or a marginal response (1.5 < MI < 2.0).

I. Discussion

The Ames test has several advantages and disadvantages as a preliminary screen for mutagenesis and carcinogenesis. The advantages are: (1) the accuracy of detecting carcinogens is high (90%), whereas the number of false positives is low (10%); (2) the test is sensitive in that it can detect mutagens in nanogram quantities; and (3) the test is rapid and inexpensive.

The disadvantages of the test are: (1) the test does not use mammalian cells; (2) the test is not responsive to certain classes of carcinogens such as metals, because of the large amount of Mg salts, citrate and phosphate in the medium; (3) certain compounds are better assayed when inducers other than Aroclor 1254 are used; (4) some compounds (especially some salts) at higher concentrations may lower the pH of the medium thus, inhibiting the growth of the bacteria; and (5) as in most bioassays, toxicity may mask mutagenicity.

The Ames test was used as a primary screen of compounds for potential carcinogenic activity. The limitations of this procedure must be understood. A compound demonstrating a positive response in the Ames test may not, in fact, be carcinogenic or even mutagenic in mammalian systems. Furthermore, a compound having a negative response may indeed be mutagenic.

BIBLIOGRAPHY

1/ Ames, B. N., J. McCann and E. Yamasaki, "Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test," Mut. Res., Vol. 31, pp. 347-364 (1975).

2/ McCann, J., E. Choi, E. Yamasaki and B. N. Ames, "Detection of Carcinogens as Mutagens in the Salmonella/Microsome Test: Assay of 300 Chemicals," Proc. Nat'l. Acad. Sci., Vol. 72, pp. 5135-5139 (1975).

3/ Purchase, I. F. H., E. Longstaff, J. Ashby, J. A. Styles, D. Anderson, P. A. Lefevre and R. R. Westwood, "Evaluation of Six Short Term Tests for Detecting Organic Chemical Carcinogens and Recommendations for Their Use," Nature, Vol. 264:624-627 (1976).

4/ Vogel, H. J. and D. M. Bonner, "Acetylornithinase of E. Coli: Partial Purification and Some Properties," J. Biol. Chem., 218, 97-106 (1956).

The following individuals will be responsible for the administrative and technical aspects of the study.

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Laurence C. K. Wong, Ph.D.
Associate Director

October 23, 1978
Estimated Starting Date

December 1, 1978
Estimated Completion Date

Study Director

Dan VanGoethem, B.S.
Assistant Biologist

January 1, 1979
Estimated Report Date

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